COMPARATIVE RANDOMISED, PARALLEL, PROSPECTIVE CLINICAL STUDY ON THE EVOLUTION OF RETINAL LESIONS DURING TREATMENTS WITH BETA-BLOCKING AGENTS + DIURETICS VS. CA \(^{2+}\) CHANNELS BLOCKERS + CONVERTING ENZYME INHIBITORS

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REZUMAT

Retinofotografia reprezinta o metoda moderna de evaluarea a microvascularizatiei retinii. Studiul nostru a evaluat timp de 12 luni evolutia leziunilor retinieni la pacientii hipertensivi aflati sub 2 tipuri de tratament antihipertensiv. Studiul randomizat a inclus 44 de pacienti aflati in stadiile I si II de retinopatie hipertensiva conform clasificarii Keith-Wagener-Barker. Pacientii au fost randomizati in grupuri de studiu si au urmat 2 asociatii diferite de tratament antihipertensiv (betablocant plus diuretic versus inhibitor de enzima de conversie plus blocante de canale de calciu). Evaluarea oftalmologica a fost efectuata prin retinofotografie la prima vizita, cu reevaluare la 6 luni si 1 an. Rezultatele au demonstrat diferente semnificative statistic in privinta evolutiei leziunilor retinieni, aceasta a fost incetinita la pacientii tratati cu combinatia inhibitori de enzima de conversie / blocanti de Ca\(^{2+}\), fata de pacientii tratati cu combinatia betablocant / diuretic.

Cuvinte cheie: retinopatie, retinofotografie

ABSTRACT

Retinophotography represents a modern method for the assessment of retinal microvascularisation. Our study evaluated the 12 months evolution of retinal lesions in hypertensive patients under two types of antihypertensive treatment. The randomised study
included 44 patients with stage I and II hypertensive retinopathy according to the Keith-Wagener-Barker classification. Patients were randomly included into study groups and received 2 different antihypertensive associations (beta blocker plus diuretic versus converting enzyme inhibitor plus calcium channels blockers). The ophthalmologic evaluation was performed by retinophotography at the first visit, with reassessments at 6 months and 1 year, respectively. The results demonstrated statistically significant differences regarding the evolution of retinal lesions, this being delayed in patients treated with the converting enzyme / calcium channels blockers combination as compared to patients treated with the beta blocker / diuretic combination, respectively.

**Keywords:** retinopathy, retinophotography

### INTRODUCTION

The interdisciplinarity of clinical examinations represents a modern trend which increases the quality of medical performance and may lead to a better evaluation of the patient's condition, as well as to a better monitoring of his/her evolution [1-3]. Modern guidelines of clinical practice recommend involving the ophthalmologist in the evaluation of the vascular patient (hypertensive, coronary diseased, etc.), the eye fundus evaluation becoming a major clinical examination component in this type of patients [4,5].

### OBJECTIVE

The objectives of our study were to evaluate, during a 12 months period, the evolution of retinal microvascular lesions under two types of antihypertensive treatment in hypertensive patients with high risk coronary heart disease.

### MATERIAL AND METHOD

The study was performed during the period August 2009 – November 2011, by collaboration between the Ophthalmology and Cardiology Clinics of the University of Medicine and Pharmacy Timisoara. The patients were selected from patients with no previous cardiology treatment who addressed the cardiology clinic for coronary heart disease symptoms. These were cardiologically evaluated and then they were referred for retinal evaluation by eye fundus examination.

All patients were subjected to a complete ophthalmologic examination. After prior pupile dilation, the stereoskopical retinophotography was performed using a Zeiss FF450 + IR fundus camera.

After the ophthalmologic evaluation, the team of cardiologists decided the further therapeutic approach.

The clinical study relied on the close cooperation between the cardiology and ophthalmology teams.

The patients diagnosed with retinopathy following the ophthalmologic examination (microanevrisms, hemorrhages or both lesion types +/- exudates) were excluded from randomisation, only those with microvascular changes, i.e. arteriovenous nicking and arteriolar narrowing, being included.

Patients were randomised into 2 groups (group A, group B). Thus, patients in group A were treated with a combination of beta blocker and diuretic (bisoprolol 10 mg + indapamide 1.5 mg), and patients in group B were treated with converting enzyme inhibitors plus Ca$^{2+}$ channel blockers (perindopril 5 mg + amlodipine 10 mg), both groups receiving the medication in the morning.

Patients were evaluated initially, then at 6 months and 1 year, respectively, by both cardiologists and ophthalmologist.
The study was approved by the local ethics committee and did not involve any company producing or promoting pharmaceutic products.

RESULTS

One hundred and fifty patients were referred for ophthalmologic assessment. Fifty of them did not have retinal lesions and 100 were diagnosed with retinopathy.

Of the 100 patients, 44 were classified with stage I/II, while other 56 had stages III/IV. The 44 stage I/II patients were further followed up at 6 months intervals.

Thus, the total number of patients who were included and randomised was 44.

Following randomisation, group A included 22 patients of whom 7 had arteriolar narrowing and 15 had arteriovenous nicking. Group B also included 22 patients, of whom 7 with arteriolar narrowing and 15 with arteriovenous nicking. Demographic data are presented in tables 1 and 2. There were no statistically significant differences between the studied groups.

Group A included 22 patients (14 men, 63.63%) with the mean age of 54±8 years. Group B included 22 patients (15 men, 68, p=NS), with the mean age of 55±7 (p=NS) years. In these tables, several paraclinical data obtained after the initial evaluation were included.

Table 1. Characteristics of patients in Group A

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>54±8 years</td>
</tr>
<tr>
<td>Sistolic blood pressure</td>
<td>163±9 mm Hg</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>95±7 mmHg</td>
</tr>
<tr>
<td>Men, %</td>
<td>14, 63.63%</td>
</tr>
<tr>
<td>Cigarette smokers, %</td>
<td>8, 36.36%</td>
</tr>
<tr>
<td>Fasting serum glucose level</td>
<td>90±8 mg%</td>
</tr>
<tr>
<td>Serum creatinine level</td>
<td>0.84 ±0.16 mg%</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>208±16 mg%</td>
</tr>
<tr>
<td>HDLc</td>
<td>31±4 mg%</td>
</tr>
<tr>
<td>K+</td>
<td>4.0±0.6 mEq/l</td>
</tr>
<tr>
<td>Na+</td>
<td>138±3 mEq/l</td>
</tr>
</tbody>
</table>

Table 2. Characteristics of patients in group B

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>55±7 years</td>
</tr>
<tr>
<td>Sistolic blood pressure</td>
<td>164±8 mm Hg</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>96±7 mmHg</td>
</tr>
<tr>
<td>Men, %</td>
<td>15, 68%</td>
</tr>
<tr>
<td>Total cholesterol level</td>
<td>229±70 mg%</td>
</tr>
<tr>
<td>LDLc</td>
<td>190±64 mg%</td>
</tr>
<tr>
<td>Cigarette smokers, %</td>
<td>9, 41%</td>
</tr>
<tr>
<td>Fasting serum glucose level</td>
<td>92±8 mg%</td>
</tr>
<tr>
<td>Serum creatinine level</td>
<td>0.87 ±0.12 mg%</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>212 ±24 mg%</td>
</tr>
<tr>
<td>HDLc</td>
<td>30±5 mg%</td>
</tr>
<tr>
<td>K+</td>
<td>4.2±0.5 mEq/l</td>
</tr>
<tr>
<td>Na+</td>
<td>139±2 mEq/l</td>
</tr>
</tbody>
</table>
The patients in group A were treated with a combination of beta blocker and diuretic (bisoprolol 10 mg + indapamide 1.5 mg), and patients in group B received converting enzyme + Ca$^{2+}$ channel blockers (perindopril 5 mg + amlodipine 10 mg) in fixed combination, with increasing doses in cases where reaching normal blood pressure values was needed.

On the initial ophthalmologic examination, the distribution of lesions in the patients included into the study (total number of patients = 44) was as follows: 14 patients had arteriolar narrowing and 30 had arteriovenous nicking. As previously stated, patients who presented any sign of retinopathy upon ophthalmoscopic examination by retinophotography (microanevrism, hemorrhages or both +/- exudates) were not included.

![Figure 1. Distribution according to the type of retinal microvascular lesions](image)

After randomisation, in both group A and group B, out of 22 patients, 7 had arteriolar narrowing and 15 had arteriovenous nicking.

![Figure 2. Incidence of retinal lesions upon initial evaluation](image)
Upon the 6 months evaluation, the data analysis detected the following incidence of retinal lesions:
In study group A, out of the 7 patients who initially had only arteriolar narrowing, 4 remained in the same evolution stage, in 2 patients the lesions progressed to arteriovenous nicking, and in 1 patient retinopathy occurred.

Also, in the study group A, out of the 15 patients in whom the initial eye fundus examination detected arteriovenous nicking, 13 remained in the same evolution stage, and 2 developed retinopathy.

In the study group B, out of the 7 patients who initially only had arteriolar narrowing, 6 remained in the same stage of evolution, and in 1 patient lesions progressed to arteriovenous nicking.

Also, in the study group B, out of the 15 patients in whom the initial eye fundus examination revealed arteriovenous nicking, 14 remained in the same stage of evolution, and 1 patient developed retinopathy.

![Figure 3. Evolution of retinal lesions after 6 months, in the studied groups](image)

After 12 months, in group A, out of the 7 patients with arteriolar narrowing only 3 remained with the same level of retinal lesions, 2 developing arteriovenous nicking, and 2 retinopathy (one with microanevrisms, another with hemorrhages). Out of the 15 patients with arteriovenous nicking, only 11 still had arteriovenous nicking after 12 months, 4 developing retinopathy (one with microanevrisms, one with hemorrhages, two with both of whom one with additional exudates).

In group B, out of the 7 patients who initially presented only arteriolar narrowing, 5 still had the same type of lesions after 12 months, and 2 had arteriovenous nicking. Out of the 15 patients with arteriovenous nicking in group A, 13 had the same type of lesions after 12 months, and 2 patients developed retinopathy (one with microanevrisms and one with hemorrhages).
These data showed that in group B the evolution of lesions was more reduced as compared to group A (4 patients in group B compared to 10 patients in group A) the calcium channel blockers + converting enzyme inhibitors combination offering a better protection in this respect as compared to the beta blocker + diuretic combination, the differences being statistically significant \( p<0.001 \).

Out of the 10 patients in group A who developed enhanced retinal lesions after 12 months, 4 developed further cardiovascular events – 2 patients of whom one with hemorrhages, one with hemorrhages and aneurisms upon ophthalmologic examination, transient ischemic attack with remitted right-sided hemiparesis, 2 patients with acute non-lethal coronary syndrome (one patient in whom the retina examination revealed hemorrhages developed instable angina pectoris, and another patient in whom the retina examination detected hemorrhages, microanevrisms and exudates was diagnosed with myocardial infarction without ST segment elevation).

Out of the 4 patients in group B who presented retinal lesions progression after 12 months, 1 patient with hemorrhages was diagnosed with instable angina pectoris.

During the clinical study, no adverse effects were recorded following the administration of medication in any of the groups.

**DISCUSSIONS**

Retinophotography during the initial visits, at 6 and 12 months, respectively, represents the best way to monitor and reveal the progression or stagnation of retinal vascular lesions during a certain time period \([6,7]\). The mere eye fundus examination allows staging according to present classes, but does not allow the dynamic assessment of lesions, but staging may be a source of errors, most commonly false negative ones, by failing to identify the aggravation of lesions \([8]\). Retinophotography allows the remote comparison of lesions and their dynamic follow up (aggravation/stagnation) and it is the most accurate and objective ophthalmologic method for the indirect evaluation of antihypertensive or anti-cholesterol treatments efficacy \([9,10]\).

The detection of aggravating retinal microvascular lesions during short time periods (6 months/1 year) imperatively
requires the reassessment of antihypertensive treatments by increasing dosage or by changing therapeutic agents.

One of the limitations in our study is the relatively short period of patient assessment (1 year). It is a well known fact that microvascular lesions have a slow tendency to aggravation in the absence of a suitable antihypertensive treatment, so we think that the assessment must be continued beyond this 1 year period.

The statistically significant differences between the two groups do not allow us to conclude on the effectiveness of the antihypertensive treatment with the 2 types of associated medications. Microvascular changes may have deep metabolic substrates, with microcirculation effects not entirely attributable to the effects of high blood pressure. There are literature references [11] to the positive reshaping effects of converting enzyme inhibitors on arteries affected by ateroma, as well as to the complex metabolic effects. The conclusions of our study are restricted to the analysis of the effects of the 2 types of associations on the retinal arterial and venous microcirculation.

CONCLUSIONS

Retinophotography is a very good assessment method for the retinal changes, being easily reproducible and offering little variation between examinations as compared to to direct or indirect ophthalmoscopy. Also, it is cost-effective reported to other methods. Retinophotography should become current practice in monitoring the evolution of persons with high blood pressure.

The detection of retinal lesions, both microvascular and, especially retinopathy lesions, should lead to a closer surveillance of patients, to prompt correction of risk factors, as well as to a more aggressive antihypertensive therapy.

The evolution of retinal lesions was significantly delayed in patients treated with the conversion enzyme inhibitors/calcium channel blockers combinations, as compared to the beta blocker / diuretic combination.

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Received for publication: 14.02.2012, Revised: 29.04.2012
MULTIDRUG-RESISTANT BACTERIAL ORGANISMS IN THE PEDIATRIC INTENSIVE CARE UNIT

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REZUMAT

Introducere: Una din principalele motive de îngrijorare a comunității medicale este creșterea prevalenței tulpinilor microbiene multirezistente și în special a tulpinilor implicate în infecțiile nosocomiale. Limitarea răspândirii acestui tip de tulpini microbiene este esențială pentru fiecare instiție de sănătate și presupune cunoașterea perfecță a fenotipurilor circulanți pentru fiecare specie microbiană. Scop: Evaluarea prevalenței și fenotipurilor de rezistență la antibiotice pentru fiecare dintre speciile microbiene izolate într-o unitate de terapie intensivă pediatrică (UTI). Material și metodă: Pe parcursul unei perioade de 18 luni (aprilie 2009 - septembrie 2010) am recoltat 2765 probe patologice provenind din departamentul de terapie intensivă a Spitalului de Urgență pentru Copii “Louis Turcanu” Timișoara. Probelor au fost fenotipate folosind sistemul VITEK 2 Compact. Rezultate: Din totalul de 2765 probe am izolat 2816 tulpini bacteriene. În cadrul acestor probe s-au izolat 1002 (35.58%) tulpini gram pozitive și 1814 (64.42%) tulpini gram negative. Din acestea, 138 (4,90%) s-au dovedit a fi tulpini MDR. Dintre acestea au fost: Acinetobacter baumannii (21.95%), S. aureus (8.47%), Klebsiella pneumoniae (7.01%), Pseudomonas aeruginosa (5.24%) și E. coli (3.70%). Concluzii: Prevalența microorganismelor MDR în departamentul de terapie intensivă este de 4,90%, majoritatea dintre ele fiind gram negative. Acinetobacter baumannii a fost microorganismul MDR cel mai răspândit (21,95%). Cuvinte cheie: MDR, Acinetobacter baumannii

ABSTRACT

Introduction: One of the main concerns in today’s medical community is the growing prevalence of multidrug-resistant (MDR) pathogenic microorganisms, especially the ones involved in nosocomial infections. Limiting the spreading of this type of microorganisms is essential for every health institution and implies a perfect knowledge of each germ’s population phenotype. Aim: Assessing the prevalence and antibiotic resistance phenotype for each of the microbial strains isolated in a pediatric Intensive Care Unit (ICU). Material and method: During an 18 month period (April 2009 - September 2010) we collected 2765
samples of pathological products from the ICU department of the Emergency Children’s Hospital „Louis Turcanu” Timisoara. The samples were phenotyped using the VITEK 2 Compact system. **Results:** From the total of 2765 samples we isolated 2816 bacterial strains. We found 1002 (35.58%) gram positive and 1814 (64.42%) gram negative strains. A total of 138 (4.90%) strains were found to be MDR. The multidrug-resistance group was composed of: Acinetobacter baumannii (21.95%), S.aureus (8.47%), Klebsiella pneumoniae (7.01%), Pseudomonas aeruginosa (5.24%) and E.coli (3.70%). **Conclusions:** The prevalence of MDR microorganisms in the ICU department is 4.90%, the majority of them being gram negative. Acinetobacter baumannii was the most prevalent MDR microorganism (21.95%).

**Keywords:** MDR, Acinetobacter baumannii

**INTRODUCTION**

Antimicrobial resistance continues to evolve and presents serious challenges in the therapy of both nosocomial and community-acquired infections [1].

In Pediatric Intensive Care Units nosocomial infections caused by multidrug-resistant bacterial organisms are increasing. They are responsible for an increase in mortality rate and for additional financial costs [2]. The rate of nosocomial infections that are caused by MDR is a good indicator of compliance with prevention control measures and antibiotic policy. It is also used to optimise the empiric treatment prescribed by clinicians. The incidence and bacterial epidemiology of nosocomial infections in children differ from those in adults, confirming the need for specific evaluation.

The types of nosocomial infections are addressed, including post-surgical wound infections, catheter-related bloodstream infections, urinary tract infections, urethral secretions, bronchoalveolar aspirates.

The purpose of this study was to measure the diversity of bacterial organisms and to assess the prevalence and the phenotype of antibiotic resistant strains.

**MATERIAL AND METHOD**

During an 18 month period (April 2009 - September 2010) we collected 2765 samples of pathological products from the ICU department of the Emergency Children’s Hospital „Louis Turcanu” Timisoara. Isolation and identification of germs were performed in the laboratory of the University Microbiology Department. All the samples were cultured on Columbia 5% sheep blood agar (bioMerieux) and selective media, like Chapmann, Mac Conkey agar (Bio-Rad). Identification was generally based on morpho-tinctorial characteristics, cultural and biochemical tests and phenotyping, using the automatic VITEK 2 compact system.

Blood samples were isolated and identified in the Microbiology laboratory of the Timisoara Clinical Emergency County Hospital.

**RESULTS AND DISCUSSIONS**

From the total of 2765 samples we isolated 2816 microbial strains. We found 1002 (35.58%) gram positive and 1814 (64.42%) gram negative strains.
Species distribution was dominated by E.coli strains (783), followed by S.aureus (744), Klebsiella pneumoniae (342), Pseudomonas aeruginosa (248), Acinetobacter baumannii (41) and other species (658).

From the total of 2816 microbial strains with nosocomial potential, 138 (4.90%) were multidrug resistant (MDR). The highest percent was reported for Acinetobacter baumannii (21.95%), followed by S.aureus (8.47%), Klebsiella pneumoniae (7.01%), Pseudomonas aeruginosa (5.24%) and E.coli (3.70%).
Table 1. Germs isolated in the intensive care unit

<table>
<thead>
<tr>
<th>ISOLATED GERMS</th>
<th>Strains</th>
<th>MDR strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>S. aureus</td>
<td>744</td>
<td>26.43%</td>
</tr>
<tr>
<td>E. coli</td>
<td>783</td>
<td>27.80%</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>342</td>
<td>12.15%</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>248</td>
<td>8.80%</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>41</td>
<td>1.46%</td>
</tr>
<tr>
<td>Other species</td>
<td>658</td>
<td>23.36%</td>
</tr>
<tr>
<td>Total</td>
<td>2816</td>
<td>100%</td>
</tr>
</tbody>
</table>

Legend: MRSA= methicilin-resistant Staphylococcus aureus
ESBL= extended spectrum beta lactamase

Figure 3. Distribution of multidrug resistant strains

All the A.baumannii strains were phenotyped, using the automatic VITEK 2 compact system, with VITEK 2 GN identification cards and AST-NO91 cards for antimicrobial sensitivity tests.

The term MDR / carbapenem resistant Acinetobacter baumannii strains was used for the first time in 1991, in a hospital in New York City [3].
Table 2. Resistance phenotypes in A.baumannii isolated from Pediatric ICU

<table>
<thead>
<tr>
<th>Species</th>
<th>Nr.</th>
<th>β lactamine resistance</th>
<th>Aminoglycoside resistance</th>
<th>Quinolone resistance</th>
<th>Another resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.baumannii</td>
<td>9</td>
<td>3 high level resistant;</td>
<td>7 resistant GANt/TGANt;</td>
<td>3 resistant-3/wild type;</td>
<td>6 TE resistant;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 carbapenem resistant;</td>
<td>2 &gt; 5 resistant phenotypes;</td>
<td>6 resistant;</td>
<td>7 SXT resistant;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8 wild type furani resistant;</td>
</tr>
</tbody>
</table>


Acinetobacter baumannii is an ubiquitous pathogen that has emerged in the last few decades as a major cause of healthcare-associated infections (HAIs) and nosocomial outbreaks. Outbreaks due MDR / carbapenem resistant Acinetobacter baumannii strains were reported, mainly in the ICU, and the treatment of these strains was considered a global problem [4, 5].

CONCLUSIONS

1. This study highlights the need to establish an antimicrobial resistance surveillance network, to monitor the trends and new types of resistance mechanisms in hospitals. A need to be identified, controlled, and, where possible, prevented so as to avoid major outbreaks.

2. The prevalence of MDR microorganisms in the ICU department is 4.90%, the majority of them being gram negative. Acinetobacter baumannii was the most prevalent MDR microorganism (21.95%).

3. The increasing frequency of carbapenem resistant Acinetobacter baumannii strains among hospitalised patients is an important problem for both microbiologists and clinicians.

4. Good hand hygiene and strict aseptic procedures remain the most important factors for infection control.

REFERENCES


ACKNOWLEDGMENTS
These data are part of the PNII 42121 project: “Molecular characterization of multidrug resistant strains, hospital or community acquired, collected from south-west Romania”.

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Received for publication: 11.01.2012, Revised: 09.03.2012
SPECTROGRAPHIC ANALYSIS OF CRYING IN NEWBORNS AND PREMATURE BABIES

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ABSTRACT

Crying in the newborn (NB) equally represents an individual biological signal as well as a symptom, a particular sign of a certain pathology or just of a disease. For the newborn, crying initially represents an individual vocal “fingerprint” in a normal or pathologic situation. A mathematical assessment of the normal domain and its pathologic variables may represent a valuable noninvasive method for the diagnosis and monitoring of postnatal neurological impairment. The present paper aims to contribute to the assessment of crying as a communication and diagnostic tool in a newborn population in the “Bega” University Clinic, Timișoara.

Key words: newborn, spectrographic analysis, premature, crying

INTRODUCTION

As crying of NB is assimilated to a communication language, it is composed of phrases, sentences and words [1]. The conversion of sounds and spectrograms, implicitly, into language is achieved according to the following criteria: crying during expiring = a word; crying cycle = a sentence; more crying cycles = a phrase [2].

The present study is retrospective and it involved the recordings and analysis of spontaneous crying of 173 newborns during the first 24 hours of life. The analysis of NB crying consisted of the evaluation of the recorded sound in the form of a sentence
and of the first subsequent word: duration, intensity, frequency and spectrogram [3].

MATERIAL AND METHOD
The present study was performed during a 4 years period, 01.05.2009-31.03.2012, in the ”Bega” Maternity Timisoara. It involved the recording and analysis of crying of 173 newborns.

The newborns included into the study were randomly selected and they were classified in full term, eutrophic NB and prematures. The group of eutrophic NB was regarded as control group.

- Description of study groups:
  - The Control Group (C)
  
  The control group includes 101 eutrophic newborns and it served as model for normality. Inclusion criteria are as follows: gestational age (GA) between 38 and 42 weeks, Apgar Index (AI) = 10, absence of maternal, fetal, anexial peripartum risk.

  This group includes 56 female and 45 male NB, and the distribution according to the year of birth is presented in Figure 1.

![Figure 1. NB distribution according to the year of birth (C)](image)

A number of 85 NB in this group were born by Cesarean section, and 16 were born naturally. The cephalic presentation occurred in 93.06% of cases. In the following paragraphs I shall graphically present the distribution of these NB according to their birth weight (BW), cranial perimeter (CP), thoracic perimeter (TP) and birth length (BL).

![Figure 2. NB distribution according to BW (C)](image)

The premature group (P)
The premature group includes NB with associated risk factors. It is composed of 72 NB. The inclusion criterion was the gestational age lower than or equal to 37 weeks. Thus, the gestational age was between 28 and 37 weeks, with a AI between 3 and 10 and the birth weight between 1200 and 3470 g. A number of 55 NB were born by Cesarean section and 17 were born naturally.
According to the year of birth there were 17 of 211 in 2009, 24 of 228 in 2010, 26 of 221 in 2011 and 5 of 39 in 2012.

I shall further present graphs showing the distribution and proportions of these NB according to gender, CP, TP and BL.
Description of the used material

In order to record the crying of newborns we used the following materials: closed incubator (Dräger Caleo), microphone, notebook.

Closed Dräger Caleo incubator - Caleo is an incubator for prematurely born babies and for diseased newborns with body weight under 5 kg and body length up to 55 cm. Inside the capsule of the Caleo device, patients receive a controlled quantity of heat and of humidity and oxygen, if required.

Microphone – The signal was recorded using a Hama Dynamic DM60 00046060 microphone.

Notebook – The recordings of crying and the database were acquired, saved and processed with an ASUS PRO55S laptop.

Data aquisition

The sound (NB crying) was recorded in a silent environment, in the closed Dräger Caleo incubator, and the microphone was placed at 15 cm from the head of the NB. The duration of one recording was 30-90
The crying of the NB was spontaneous – unprovoked, and the recording was performed during the first 24 hours after birth. The sound was recorded and saved using the Neonat application. The application, suggestively named Neonat, was created to run under the Windows operating system, in order to record NB crying.

![Figure 9. The main menu of the application](image)

![Figure 10. Form](image)

![Figure 11. Recording window](image)
Data processing

The data collected during the study were processed by data mining techniques, statistically using SPSS 17.0, but also spectrographically. For processing the recorded sound we used the following software applications: WavePad Sound Editor, Speech Analyzer 3.0.1 and Sigview v2.4.0.

Data Mining – represents the observational analysis of (often very large) data sets with the purpose to find correlations and to summarise data into collections which are both meaningful and useful to the data owner [4,5].

Database (DB) – The database was constructed in an Excel file by sequential data entries from the records (Observation chart) of each patient.

The data were organised in a database of the SPSS 17.0 software being processed by descriptive statistics (the elements previously described), by testing statistical significance, analysis of differences – using the T – Student test, and we established the p value under or equal to 0.05 (95% confidence interval – CI) as the threshold of statistical significance.

RESULTS

• Duration of a sentence

The analysis of crying revealed a medium, maximal and minimal duration of a sentence as shown in Table 5, and the distribution inside the studied groups is shown in Figure 12 [6].

Table 1. Duration of a sentence

<table>
<thead>
<tr>
<th>Groups</th>
<th>C</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>101</td>
<td>72</td>
</tr>
<tr>
<td>Average</td>
<td>11.87</td>
<td>9.29</td>
</tr>
<tr>
<td>Std.Dev.</td>
<td>2.777</td>
<td>1.690</td>
</tr>
<tr>
<td>Minimum</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Maximum</td>
<td>23</td>
<td>14</td>
</tr>
</tbody>
</table>

Figure 12. Duration of a sentence

Duration of a crying sentence is about 10-13 sec for the control group, the other group showing lower average durations, i.e. 8-10 sec.

• Number of words/sentence

In the control group the number of words per sentence varied from 4 to 18 with an
average of 9.45 and a standard deviation of 3.381. Prematurely born babies produced an average number of words of 7.24 with a standard deviation of 2.861, a minimum number of 3 words/sentence and a maximum of 17 words/sentence.

Figure 13. Number of words/sentence

- **Average intensity, maximal intensity and spectrogram analysis**

**Control group (C)**

The analysis of NB crying in the control group revealed an average intensity (VU) of 365.287 units (U) with variation between 11-3106 U. The average values of the minimum VU being around 11 U, and the maximum VU being around 1483 U. From the maximal intensity analysis (PP) we found that the average value was at 1659.973 U with variations between 44-12952 U. The average values of the PP minimum were around 343 U, and for the maximum PP, 4379 U. Regarding the frequency spectrum we found average values of 157.371 U with maximum values at 11582.54 U.

The figure below brings additional information on the graphic structure of the previously described sound:
The group of premature NB (P)
There were 72 premature newborns included into the study. They presented significant changes in crying intensity and frequency spectrum as compared to C. Thus, the average VU had variations between 16 and 3664 U with an average around the value of 1092.665 U, and the sharp sounds of these NB were situated in the range 1285 – 10084 with an average of 4538.97 U. So, the average frequency spectrum was 496.827 U, and the average maximum values were around 37915 U [7].

The figure below shows crying of premature NB. This is different both in quality and quantity from that of an eutrophic NB.

Quantitatively, I am referring to the duration of a sentence which is in average 12 sec in an eutrophic NB and only 9 sec in a premature NB. In this category I am also including the number of words per sentence, this being seven as an average, with two units lower than that expressed by NB in the C group.
- *The analysis of the first word*

The first word is defined as the sound expressed by a NB during the first expiration after a crying sequence. This, as the sentence, is characterised by duration, intensity and frequency.

In the control group, the first word analysis revealed an average intensity of *641.68 U* with variations between U and 1241 U. Regarding the sharp sounds, these NB presented values between 1032 U and 3637 U with an average of *2449.202 U*, and regarding frequency, the average was *249.366 U*, with maximum values reaching 8008.891 U.

The first word in the studied sentences of premature NB presented the following characteristics: average VU of 1606.966 with variations between 188-2921 U, average PP at 5861.883 U which varied between 2574–8128 U, and average frequency of 600,717 U with maximum value of 20570.
CONCLUSIONS

Unprovoked crying in the eutrophic NB is organised, well structured. The sentence is formed of words with intensity and duration decreasing from the onset of crying to the end of the sentence [8].

Qualitatively, the two forms of crying differ in the structure of the sentence. The C group includes words which decrease in intensity and duration correlated to the time axis in contrast to the crying of premature NB which includes words with variable duration, without expressing any clear increase/decrease pattern in correlation with time. Also, in each word, the intensity is increased, in plateau, the transition to pause being done abruptly.

The duration of a crying sentence is around 10-13 sec in the reference group, and 8-10 sec for premature babies.

During all this time, the NB may ”speak” around 7-11 words in the case of eutrophic NB and 4-6 words in the case of premature NB.

Statistically, between the 2 groups (M*P) we found extremely significant differences in: the average intensity (p=0.00071, p<0.001), the maximal values of the average intensity (p=0.00028), the maximum intensity (p=0.00026), the maximal values of the maximum intensity (p=0.00015), the frequency spectrum (p=0.0008) and maximal values of the frequency spectrum (p=0.00027). Highly significant differences were found in the case of the minimum values of maximal intensity (p=0.0036).

Comparing the values obtained after analysis of the first word of eutrophic NB with those of NB in the C group we did not find significant differences. On the other hand, the comparison between the reference and premature NB revealed average intensities (VU), sharp sounds (PP), but also significantly higher frequencies for premature NB. These differences (significantly higher values) were also recorded at the time of comparison between the group of NB in a high risk category (GP) and the C group [9].

The control group – Unprovoked crying of eutrophic NB, its sentences in particular, are situated around an average intensity of 365.287 U, with sharp sound values at 1659.973 U and an average frequency of 157.371 U.

The P group – Premature newborns, when compared to eutrophic ones, presented extremely significant statistical differences in intensity and frequency spectrum. Thus, the crying of premature NB presents increased intensities (1092.665 U), covering...
a high range of the frequency spectrum (496.827 U)a.

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Received for publication: 04.01.2012, Revised: 12.03.2012
ACTUALITIES IN DIAGNOSIS AND MONITORING OF URINARY INFECTIONS CAUSED BY ANTIBIOTIC RESISTANT BACTERIA

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ABSTRACT

In the present context of rapid increase in the prevalence of resistant Enterobacteriaceae strains, the need to use molecular biology methods becomes increasingly stringent, these being much more sensitive than phenotypic testing and the epidemiologic surveillance and monitoring of CTX-M producing strains are more important for establishing therapeutic strategies for the revision of empiric treatment protocols. Our study is new for our country involving molecular analysis of plasmid genes with great community dissemination potential.

Keywords: urinary infections, antibiotic resistance, extended spectrum beta-lactamases

INTRODUCTION

The resistance of microorganisms to antibacterial products is a priority for public health systems both nationally and globally. Infections caused by resistant microorganisms determine high morbidity and mortality levels due to therapeutic failures and rising costs for medical care. Characteristics and dimensions of the antibiotic resistance phenomenon were identified by laboratory methods and demonstrated by unfavorable clinical evolution of patients [5,10,12].
The main causes of increasing resistance to antibiotics are inappropriate use and invalid prescription of these products by misinterpretation of symptoms, uncertain diagnosis and perceived expectations of the patient, too long/too short duration or inappropriate dosage, self-medication, use of antibiotics in the veterinary sector, poor arsenal of diagnostic measures, drugs and vaccines, inadequate drug supervision and control [3,7].

Difficulties in the treatment of infectious diseases occur more often in infections caused by strains producing ESBLs (Extended Spectrum Beta Lactamases) - enzymes that develop resistance to extended-spectrum antibiotics [15].

Presently, the emergence of Enterobacteriaceae strains producing ESBLs is a strong threat, in terms of effectiveness of antibiotics use in the therapy of infections [8].

Analyzing literature data we highlight a number of specialized phenotypic and genotypic methods to determine the ESBLs-producing strains [14,16].

The detection of the specific type of beta-lactamase is not possible by routine tests (disc - diffusion, chromogenic tests etc.). The combination of several types of beta-lactamases in the same microorganism makes it more difficult to detect correctly. Studies made by other researchers demonstrate the importance of molecular biology techniques in the detection of ESBLs [1,6,11].

The correct detection of ESBLs producing strains, CTX-M remains a challenge for the microbiology laboratory and is very important to prevent clinical failure due to inadequate antibiotic therapy.

The purpose of the proposed study was to identify phenotypes of resistance among circulating strains of E.coli in our geographical area and to detect the molecular mechanism of resistance, by detecting the presence of resistance markers (beta-lactamase) using phenotypic and molecular methods.

**MATERIAL AND METHODS**

For the study, were collected 118 urine and stool samples from people with a diagnosis of urinary tract infection (UTI). The study was organized under the Scientific Research Institute of Mother and Child Health Care, Diagnostic Center "Modus Vivendi". Researches were conducted in the laboratory of Center epidemiology of the highly contagious disease and Biosafety National Center for Public Health, in collaboration with the bacteriology laboratory of the hospital Bichat - Claude Bernard, Paris, France.

Urine samples were inoculated on media for differential diagnoses: Endo, blood agar, hypersaline agar with egg, enterococci agar, Mueller Hinton, Sabouraud. For the research, only strains isolated from significantly positive urine samples (≥ 105 CFU / ml) were selected. Strains of E. coli were identified by gender and / or species by conventional biochemical tests (Voges - Proskauer test, indole test, urea, citrate, fenilalanindezaminaze etc). Confirmatory tests were performed using API 20E galleries (BioMérieux, France). The subsequent phase of the research included the susceptibility testing of E. coli strains to antimicrobial preparations using phenotypic methods (Kirby-Bauer disk diffusion test, synergy test - bidimensional distribution of the two discs with antibiotics) and molecular biology (polymerase chain reaction, sequencing, multiplex PCR, Rep - PCR).

The evaluation of results was performed in accordance with recommendations of the Clinical and Laboratory Standards Institute guidelines (CLSI M100-S20, 2010). The E. coli ATCC 25922 strain was used as a reference strain for antibiotic susceptibility testing on the culture media used.
The susceptibility testing of E. coli strains to antimicrobial preparations included the following classes of antibiotics: beta-lactam antibiotics - amoxicillin (AMX), ticarcillin (TIC), amoxicillin / clavulanate (AMC), cefotaxime (CTX), ceftazidime (CFT), cefotixin (FOX), cefepime (CFP), ertapenem (ETP), aminoglycosides - gentamicin (GM), amikacin (AN), kanamycin (K); fluorchinolone - nalidixic acid (NA), ofloxacin (OFX), ciprofloxacin (CIP), tetracycline - tetracycline (TE) and sulfonamides - cotrimoxazole (sulfamethoxazole trimetoprim) (SXT).

RESULTS AND DISCUSSIONS

Microbial resistance to antibiotics is a global public health problem and it is largely caused by the inappropriate use of antibiotics. The antibiotic resistance phenomenon in Europe is continuously growing. The term of urinary tract infections (UTI), refers to the infectious diseases commonly encountered in medical practice which record a high incidence and prevalence with medical and economic consequences. The strains involved in the etiology of UTI are part of the epidemiological and bacteriological studies in recent years [2,13].

The results of studies show that E. coli, germ belonging to the normal microflora of the gastrointestinal tract, under certain conditions becomes significantly resistant to antibacterial products and can cause intestinal and extraintestinal infections (including urinary infections) [4,14,15].

According to studies concerning the prevalence and etiological structure of urinary infections, in 95% of cases these are caused by agents of the Enterobacteriaceae family (with 80-95% of cases caused by E. coli, and less frequently by Proteus spp. or Klebsiella spp.) and the remaining 5% by Pseudomonas aeruginosa, staphylococci, yeasts of the genus Candida, etc. [7,9,14-16].

The results of biochemical screening and confirmation tests for Enterobacteriaceae, revealed that from all the strains isolated in patients with UTI, E. coli were predominant (92.9%).

E. coli strains isolated from patients with UTI were tested to identify the degree of resistance to five classes of antibiotics. The resistance to antibiotics determined by the Kirby-Bauer disk diffusion method revealed that the studied E. coli strains, isolated from people with ITU have a high level of resistance, including to beta – lactam antibacterial products. Also, the beta-diffusion method does not provide sufficient data to assess the level of resistance and a correct monitoring of antibacterial therapy. Synergy tests were used to elucidate one of the mechanisms of antibiotic resistance of E. coli strains - the presence of beta-lactamases. This test uses a beta-lactamase inhibitor, clavulanic acid usually in combination with an oximino-cephalosporin such as ceftazidime, cefotaxime, ceftriaxone. The antibiotic discs were placed in such a way to observe the synergy between discs with amoxicillin / clavulanic acid and the cephalosporin III generation (CG3). The location sequence of discs with appropriate antibiotics was the following: TIC - FOX - CFP - AMX - GM-CFT - AMC - CTX.

As a result, we determined that out of 118 E.coli strains isolated from urine cultures, 91 strains of E. coli have resistance to ticarcillin, 49 strains – to amoxicillin, 26 strains – to cotrimoxazole, all 21 strains – to nalidixic acid and ofloxacin, 18 strains - to ciprofloxacin and 7 strains of E. coli to other antibiotics (Figure 1).
Beta-lactamase producing E. coli strains were preserved in an environment containing ox-heart broth and 10% glycerol and then frozen at -80°C to be further tested by molecular biology techniques.

The adequate treatment protocol requires rapid and accurate identification of antibiotic resistant strains using molecular biology techniques.

The determination of resistance profiles showed resistance to the following groups of antibiotics: aminoglycosides - gentamicin (72%), fluoroquinolone - nalidixic acid (89%) and sulphonamides - cotrimoxazole (72%). Strains isolated from the stool are also polyresistant. They are resistant to the same classes of antimicrobials as ESBLs E. coli isolated from urine: 53% to aminoglycosides (gentamicin), 56% to fluoroquinolones (ciprofloxacin), 44% to sulphonamides (cotrimoxazole).

ESBLs producing E. coli strains isolated from urine samples were of the CTX-M type, namely: a type CTX-M-1 strain, 3 type CTX-M-14 strains, other 3 type CTX-M-15 strains. ESBLs E. coli strains found in stool samples were predominantly of CTX type and only one enzyme of SHV type. As opposed to urinary strains, those were relatively more varied, namely one CTX-M-1, 2 CTX-M-3, 8 CTX-M-14 and 3 CTX-M-15 strains, respectively.

The ability to clone ESBLs E. coli strains detected in urine and stool was investigated by Rep-PCR method, only four strains of E. coli containing ESBLs in both urine and feces (U + / F +). In three of four ESBLs E. coli strains U + / F + tested, a Rep-PCR similar profile was present.

After determining the phylogenetic group of urinary strains the results showed that 58.5% of the ESBLs E. coli strains belong to the B2 group, 27.9% to the A group, 12.7% to the D group and 0.9 to other groups. The strains detected in the faeces have the following phylogenetic diversity: 53.4% - group A, 23.1% - group B2 and group D, and 0.4 - other groups (Figure 2).
Phenotypic and genotypic monitoring of antibiotic resistance markers in human populations is a constitutive key in the national surveillance system for the antimicrobial resistance phenomenon, following to be developed in Moldova as a part of state public health surveillance.

**CONCLUSIONS**

1. The used molecular biology methods involved relatively high costs, but provided reproducible results in a very short time, detecting in E. coli strains isolated from urine and coprocultures the ESBLs – beta-lactam antibiotics resistance marker.

2. The circulation of CTX-M type, ESBLs secretor E. coli strains within the Republic of Moldova demonstrates the complexity of the resistance mechanisms, which requires constant monitoring measures of the spread of these strains, because therapeutic options are limited and can lead to fatal evolutions. A constant monitoring of the resistance phenomenon and the molecular analysis of pathogens are required.

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Received for publication: 22.01.2012, Revised: 15.03.2012
IDENTIFICATION OF ANTIBIOTIC RESISTANCE PHENOTYPES IN ESCHERICHIA COLI ISOLATES FROM URINARY TRACT INFECTIONS

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2. “Victor Babeș” University of Medicine and Pharmacy Timișoara, Department of Microbiology

ABSTRACT

Aims: The aim of our study was to determine the prevalence of Escherichia coli strains, isolated from urine cultures of patients hospitalised in the Obstetrics and Gynecology department, and their resistance patterns. Methods: Identification of germs and extensive antimicrobial tests (by dilution antimicrobial susceptibility tests) were performed with the help of the automatic Vitek2 System (bioMérieux France). For extended spectrum beta-lactamases (ESBL) detection we performed the Vitek ESBL test (AST-GN27 cards), which includes cefotaxime and ceftazidime, alone and in combination with clavulanic acid. Results: From 313 urine samples we isolated 170 microbial strains with nosocomial potential, of which 130 (76.47%) were Escherichia coli. The highest percentage was noticed in the case of penicillin resistant Escherichia coli strains and I-II generation cephalosporins. Most of these
strains associated other resistance phenotypes as well. **Conclusions:** The high prevalence of *Escherichia coli* strains resistant to antimicrobial agents in our study, enforces a proper surveillance of medical staff and a rational policy in prescribing antibiotics.

**Keywords:** urine cultures, resistance phenotype, *Escherichia coli*

### INTRODUCTION

The urinary tract is one of the most common sites of nosocomial infection, and most of these hospital-acquired infections occur in patients who have undergone urologic procedures (frequently catheterization). Bacteria that produce catheter-associated urinary tract infection can be acquired from the patient’s fecal flora, or by cross-infection (transfer of bacteria from patient to patient by hospital personnel).

The incidence of catheter-associated urinary tract infection depends on the method and duration of catheterization [1].

Hospital-acquired urinary tract infections may be associated with resistant organisms and the choice of therapy is dependent on antibiotic sensitivity tests [2].

The therapy of both nosocomial and community-acquired infections is affected by the continuing evolution and challenges presented by antimicrobial resistance [3].

*E. coli* is an important human pathogen. It is the bacterial species most frequently isolated from biological samples. In acute urinary tract infections, *E. coli* is the causative organism in 70–80% of cases and in chronic, persistent infections 40–50% of cases are caused by this agent [4, 5].

The development of resistance in Gram-negative bacilli includes the recognized decline in their susceptibility to third-generation cephalosporins and to fluoroquinolones. The extended-spectrum β-lactamases are generally encoded by mobile genes that can be highly prevalent among some enterobacteria, such as *E. coli* and *Klebsiella pneumoniae* [6].

### MATERIAL AND METHODS

During a period of one year we collected 313 urine samples from patients hospitalised in the Obstetrics and Gynecology Department of the Timișoara Clinical Emergency County Hospital. This study was approved by the local Ethics Committee. The patients were included in our study only after obtaining their informed consent and based on the norms of ethics generally applicable regarding the human individual protection according to the stipulations of Law no. 206/2004. The 98/44/EC and 2001/20/EC Norms of the European Parliament and the Council of the European Union regarding the protection of personal data and the deployment of good clinical practice have been respected.

From 170 positive urine cultures we isolated 130 *Escherichia coli* strains. Identification was performed at the University “Victor Babeș” Timișoara, in the Microbiology Department's Laboratory.

Quantitative bacterial culture of each urine specimen was performed by inoculating culture media (Columbia agar supplemented with 5% sheep blood and Mac Conkey agar) with a measured amount of urine using calibrated loops designed to deliver a known volume.

The identification of germs was based on colonial appearance and biochemical characteristics. Final bacterial identification was performed using the automatic Vitek2 Compact System (bioMerieux France). Extensive antimicrobial tests (by dilution antimicrobial susceptibility tests) were performed with the help of the automatic Vitek2 System. For extended spectrum beta-lactamases (ESBL) detection we performed the Vitek ESBL test (AST-GN27 cards), which includes cefotaxime and ceftazidime,
alone and in combination with clavulanic acid.

For testing we used the following antimicrobial agents: ampicillin, amoxicillin/clavulanic acid, piperacillin, piperacillin/tazobactam, cefazolin, cefoxitin, cefotaxime, ceftazidime, cefepime, imipenem, amikacin, gentamicin, ciprofloxacin, norfloxacin, tetracycline, nitrofurantoin, trimethoprim/sulfamethoxazole.

**RESULTS**

We isolated 170 strains with nosocomial potential, of which 130 were represented by Escherichia coli strains (76.47%).

![Figure 1. Isolated germs](image)

**Table 1. Resistance to β-lactam antibiotics in Escherichia coli isolates**

<table>
<thead>
<tr>
<th>β-lactam antibiotic</th>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>35</td>
<td>26.92%</td>
<td>-</td>
</tr>
<tr>
<td>Amoxicillin/clavulanic acid</td>
<td>84</td>
<td>64.61%</td>
<td>6</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>35</td>
<td>26.92%</td>
<td>-</td>
</tr>
<tr>
<td>Piperacillin/tazobactam</td>
<td>84</td>
<td>64.61%</td>
<td>13</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>35</td>
<td>26.92%</td>
<td>49</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>130</td>
<td>100%</td>
<td>-</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>120</td>
<td>92.3%</td>
<td>-</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>120</td>
<td>92.3%</td>
<td>-</td>
</tr>
<tr>
<td>Cefepime</td>
<td>126</td>
<td>96.92%</td>
<td>-</td>
</tr>
<tr>
<td>Imipenem</td>
<td>130</td>
<td>100%</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 2. Resistance to other antibiotics in Escherichia coli isolates

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Amikacin</td>
<td>124</td>
<td>95.38%</td>
<td>-</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>101</td>
<td>77.69%</td>
<td>-</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>103</td>
<td>79.23%</td>
<td>-</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>103</td>
<td>79.23%</td>
<td>-</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>123</td>
<td>94.61%</td>
<td>-</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>104</td>
<td>80%</td>
<td>-</td>
</tr>
<tr>
<td>Trimethoprim/sulfamethoxazole</td>
<td>112</td>
<td>86.15%</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3. Resistance phenotypes in Escherichia coli isolates

<table>
<thead>
<tr>
<th>Phenotypes</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wilde type</td>
<td>31</td>
<td>23.84%</td>
</tr>
<tr>
<td>Low PASE</td>
<td>28</td>
<td>21.53%</td>
</tr>
<tr>
<td>High PASE</td>
<td>28</td>
<td>21.53%</td>
</tr>
<tr>
<td>Low PASE+Q</td>
<td>3</td>
<td>2.31%</td>
</tr>
<tr>
<td>Low PASE+AG</td>
<td>1</td>
<td>0.76%</td>
</tr>
<tr>
<td>Low PASE+AG+FT</td>
<td>3</td>
<td>2.31%</td>
</tr>
<tr>
<td>Low PASE+AG+Q</td>
<td>3</td>
<td>2.31%</td>
</tr>
<tr>
<td>Low PASE+AG+SXT+FT</td>
<td>10</td>
<td>7.69%</td>
</tr>
<tr>
<td>Low PASE+SXT+FT+TE</td>
<td>2</td>
<td>1.53%</td>
</tr>
<tr>
<td>High PASE+Q+FT+TE</td>
<td>3</td>
<td>2.31%</td>
</tr>
<tr>
<td>High PASE+Q+SXT+FT</td>
<td>6</td>
<td>4.61%</td>
</tr>
<tr>
<td>High PASE+AG+Q+FT+TE</td>
<td>2</td>
<td>1.53%</td>
</tr>
<tr>
<td>ESBL+AG+Q</td>
<td>10</td>
<td>7.69%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>130</td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

Legend: ESBL-extended spectrum beta-lactamase, Low PASE- low level penicillinase, High PASE-high level penicillinase, CASE-cephalosporinase, AG-aminoglycosides, Q-cross resistance to all quinolones, SXT-trimethoprim-sulphamethoxazole, FT- nitrofurantoin, Te-tetracycline.

**DISCUSSIONS**

E. coli strains are associated with healthcare-associated infections and community-acquired urinary tract infections, in nearly all countries over a longer period of time. In 2007, 23 of 28 countries reported 50% or even higher resistance to aminopenicillins. Therefore, these agents can no longer be rendered as therapeutical options for the empirical treatment of these infections. A significant increase in the resistance to third generation cephalosporins was reported in 19 of 26 European countries from 2001 to 2007 [7, 8].

In our study, 10 (7.69%) of 130 E. coli strains, were ESBL producing, 39 strains
(30%) were high level penicillinase producing and 47 strains (36.15%) were low level penicillinase producing.

The ESBLs are beta-lactamases capable of conferring bacterial resistance to the penicillins, first-, second-, and third-generation cephalosporins, and aztreonam (but not to the cephamycins or carbapenems) by hydrolysis of these antibiotics, and which are inhibited by beta-lactamase inhibitors such as clavulanic acid or tazobactam. The most common extended-spectrum phenotypes arise from point mutations in the blaTEM, blaSHV, or blaCTX genes resulting in alterations of the primary amino acid sequence of the enzyme [9]. Since these genes are generally found on plasmids, many of the organisms that harbor ESBLs also are resistant to other classes of antibiotics, such as aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol, and sulfonamides [10]. Many strains were multiresistant, being sensitive only to imipenem [11].

In this study, all ESBLs have been associated with other resistance patterns like: cross resistance to quinolones and aminoglycosides. We did not isolate any strain with imipenem or cefoxitin resistance.

There is a considerable geographical difference in the occurrence of ESBLs in the European countries. In a SENTRY worldwide surveillance program report, ESBL phenotypes were detected in 1-8% of E. coli strains [10].

Fluoroquinolones resistance has increased in 24 from 26 countries since 2001, and in 2007 the proportion of resistant isolates ranged from 7% to 40% (with ten countries reporting more than 25% fluoroquinolones resistant E. coli strains) [7, 8].

The occurrence of aminoglycosides resistance in E. coli ranged from 25% to 38%, with 17 of 26 countries reporting a 10% to 25% resistance and with only Romania reporting more than 25%. A significant increase was reported from 2001 to 2007 in 16 of 26 countries [7, 8].

Our E. coli strains were resistant to: aminoglycosides 22.3%, quinolones 20.76%, tetracycline 5.38%, trimethoprim/sulfamethoxazole 13.84% and nitrofurantoin 20%.

**CONCLUSIONS**

1. The routine detection of the β-lactam resistant phenotypes in E. coli clinical isolates is particularly important because of therapeutic problems related to the acquired resistance in this species.
2. No carbapenemase producing E. coli strains have been reported.
3. Proper surveillance of medical staff and a rational policy in prescribing antibiotics in hospitals are therefore mandatory.

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Received for publication: 17.01.2012, Revised: 03.04.2012
THE EFFECTS OF TOBACCO SMOKING ON THE HEALTH OF ADOLESCENTS AND YOUNG ADULTS

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REZUMAT

Efectele pe termen scurt ale fumatului în rândul tinerilor le includ pe cele asupra aparatelor respiratorii și cardiovascular, nivelul de antrenament, efectele dependentei de nicotină, efectele consumului de tutun în scopul controlului greutății. Consecințele pe termen lung asupra stării de sănătate ale fumatului apar în timpul vieții de adult.

Cuvinte cheie: tutun de fumat, efecte asupra sănătății asupra tinerilor

ABSTRACT

The short term effects of cigarette smoking among young people include those upon the respiratory and cardiovascular apparatus, the fitness level, the effects of nicotine addiction, the effects of tobacco use for weight control purposes. Long term health consequences of tobacco consumption occur during adult life.

Keywords: tobacco smoking, health effects upon young people

The consequences of tobacco consumption on the health of adults were extensively studied starting with the 1950s [1]. Among young people, the short term effects of tobacco smoking include those upon the respiratory apparatus as well as upon other apparatus, the effects of nicotine addiction, the risk of concomitant use of other substances. Long term consequences of tobacco consumption occur during adult life.

1. Epidemiological evidence of tobacco smoking effects upon the respiratory system

Numerous study reviews demonstrated that active tobacco smoking during childhood and adolescence has the potential to decrease the rate of pulmonary growth and to reduce the the pulmonary functional capacity, thus increasing the risk of chronic obstructive lung disease during adulthood [2].

The assessment of the effects of smoking upon the pulmonary function in children and young adults requires an understanding of gender normative differences in growth models and at the age when the maximal level of the pulmonary function is reached. The maximal pulmonary function is reached after reaching the maximal height and it occurs later in boys than in girls [3,4].
The cardinal symptoms indicating the existence of lesions of the respiratory system are coughing, sputum production, wheezing and dyspnoea. Epidemiological studies based upon questionnaires applied to regularly smoking adolescents and young adults (those smoking at least once a week) applied between 1960 – 1980 and including thousands of responders, offer conclusive evidence that tobacco smoking is associated to cough and phlegm in the throat. Numerous studies found that wheezing and dyspnoea were significantly associated to tobacco smoking [5,6].

The effects of smoking in adolescents may be confounded with a history of passive smoking if the adolescent's parents smoke in his/her presence [7].

Numerous transversal studies performed in adults showed that smokers have a reduced level of the pulmonary function as compared to persons who never smoked [8]. Longitudinal studies showed that smoking accelerates the age related decline of the pulmonary function. The most conclusive evidence were obtained by spirometric measurement of the pulmonary function.

One of the most valuable measurements obtained through spirometry is the forced expiratory volume per second (FEV₁). FEV₁ increases in parallel with the pulmonary growth and development during childhood and adolescence [9,10]. In persons who never smoked, FEV₁ starts to decrease after the 3rd-4th decade of life. In smokers, the decline of FEV₁ begins at young ages in a more accelerated pattern as compared to non-smokers. When individuals quit smoking, FEV₁ will gradually reach the specific age related decline [9].

The results of a study performed in the Netherlands [11] showed that in women, FEV₁ reaches a plateau at the age of 15 years, while in men, FEV₁ continues to increase until around the age of 20 years. Nevertheless, in average, women have a longer plateau, their pulmonary function starting to decrease around the same age of 25 years as in men. Both current as well as cumulative smoking were significant predictors of FEV₁ in men, with decline measurement differences – 85 ml per 10 cigarette packages/year for cumulative smoking. Even though in women no effect of smoking upon FEV₁ was found, no significant gender differences were found in the effect of smoking, and also the number of young female smokers was low. Smoking was associated to a lower FEV₁ level both in men and in women. The investigators observed that the smoking effect magnitude in this young group was higher than the one found in cohorts older than 35 years in studies elsewhere.

Recent studies demonstrated the relation between current smoking and the difficult to treat asthma in young and middle aged adults. Chaudhuri et al. [12], in a randomized placebo controlled study, performed in groups in whom 40 mg prednisolone or placebo were given, found a significant improvement of the FEV₁, of the maximal expiratory volume measured in the morning and of the asthma control score after the administration of prednisolone in persons who never smoked. No improvement was observed in asthma patients who smoked. Ex-smokers had a significant improvement of PEF in the morning and in the evening, but not of the FEV₁.

As smoking was proved to alter the immune inflammatory response, one of the plausible effects would be the increase of the frequency and severity of respiratory infections in smokers. In a study conducted in Great Britain on 2885 children aged between 12 and 13 years [13], children who reported smoking regularly had a poorer state of health and significantly more medical leaves of absence. In another study performed by Townsend [14] in adolescents, smokers reported a higher prevalence of
health problems as compared to non-smokers.

2. Epidemiological evidence of the effects of smoking on the cardiovascular system

In adults, smoking causes coronary heart disease, atherosclerosis in the peripheral blood vessels and stroke [2]. Even though these pathologic conditions are rarely encountered in children and adolescents, autopsies performed in adolescent and young adult smokers, during the wars in Korea and Vietnam showed that, in smokers, the atherosclerotic process starts as early as the adolescence, and becomes clinically significant in early adulthood [15,16].

When comparing adolescents exposed to cigarette smoke to other non-exposed adolescents, an increase in the level of oxidative stress and lower antioxidant levels were found in exposed adolescents [17]. In a study performed by the Pathobiological Determinants of Atherosclerosis in Youth Research Group [18] on 390 young persons aged between 15-34 years, who died by violent causes, after controlling for lipid levels, age and race, by a multiple regression analysis significant associations were found between smoking and atherosclerosis of the abdominal aorta.

The ultrasound assessment of vascular reactivity in the brachial artery was the first documented evidence of the direct effect of tobacco exposure on the cardiovascular system in young people [19]. The vascular reactivity determined by this mechanism is considered as an endotracheal health index. The adverse effects on active and passive smokers were demonstrated by measuring the endothelial function. The endothelial disfunction was demonstrated in current young smokers with a dose-response relation, but also in young persons exposed to passive smoking.

In a study performed in young Japanese, with an average age of 32 years, an endothelial disfunction was shown as a response to active or passive smoking. Both the endothelial disfunction and the exposure were correlated to the plasma levels of 8-isoprostanate, a measure of the oxidative stress [20].

The evidence associating smoking to dyslipidemia cover both active and passive smoking. At present, there are several studies connecting passive smoking exposure to lipidic abnormalities in children. A cohort study conducted in twins [21] found lower HDL-cholesterol levels in children with chronic exposure to passive smoking, at the initial moment, and this difference persisted in time, after controlling for other cardiovascular risk factors, overweight and family histories of cardiovascular diseases [22]. A study conducted in highschool children practicing sports, used the measurement of plasma cotinine as passive smoking exposure marker and found lower HDL-cholesterol levels in those with a level indicating cotinine exposure [23]. Similarly, in a transversal study on 104 children, the lower HDL-cholesterol level was associated to family cohabitation with at least one smoker [24].

In young people, smoking was associated to the alteration of serum lipids profiles, thus increasing the risk of cardiovascular diseases. In a meta-analysis on studies including children who smoked, Craig et al. [25] found that smoking increased the levels of HDL-cholesterol with 4%, triglycerides with 12% and VLDL with 12%. HDL-cholesterol levels were decreased with 9%. These results are comparable but with a higher magnitude as compared to those observed in adult smokers.

The relation between tobacco consumption and insulin resistance was studied with interest during recent years [26,27]. In the CARDIA study, tobacco consumption was associated to the gradual onset of glucose intolerance: the continuous tobacco
consumption predicted the highest probability of glucose intolerance, while previous exposure to passive smoking was associated to a lower probability [28]. In a meta-analysis on the smoking-diabetes mellitus relation including 1.2 million persons, the 60% increase in the risk of type 2 diabetes mellitus in heavy smokers, and lower but still significant increases in the risk of the type 2 diabetes mellitus in lighter smokers were confirmed [29]. These studies included subjects of various ages, between 16 and 60 years at the starting point, but were especially presented for adolescents and young adults.

3. Epidemiological evidence on the effects of smoking upon the fitness level
It has been proven that in the case of athletes, smoking also alters the physical fitness level, both concerning performance and endurance. Smoking decreases the oxygen transport capacity and increases the cardiac and the basal metabolic rates, changes which counteract the benefits of physical activity [30].

A study [31] conducted on 6500 nineteen year old soldiers showed that soldiers who smoked ran a significantly shorter distance in 12 minutes, and an 80 m sprint lasted significantly longer than in their non-smoking colleagues. In the 16 km endurance running, smokers were significantly slower than non-smokers.

Young adult smokers have slight but chronic physiological cardiovascular alterations, including decreased performance in physical exercises upon stepper tests and an abnormal cardiac rate response [32].

4. Epidemiological evidence of nicotine addiction symptoms and diagnosis
Studies suggest that adolescents report addiction symptoms even at low cigarette consumption levels [33,34]. The difference between nicotine sensitivity in adolescents and adults was also reported in animal models [35].

DiFranza et al.[36] concluded that the onset symptom of tobacco addiction occurs when adolescents reach the average smoking level of 2 cigarettes a week. Even adolescents who only smoked once or twice throughout their entire life reported an average of 1.3 symptoms in HONC (Hooked on Nicotine Checklist) [37].

Kandel and Chen [38] examined a diagnostic tool for establishing the nicotine addiction diagnosis based upon the National Household Survey on Drug Abuse questionnaire. They reported that adolescents, as compared to adults, had addiction criteria even at lower levels of tobacco consumption. Some researchers suggested that these differences found in the two age groups reflect a higher nicotine sensitivity in adolescents as compared to adults.

The reported prevalence of nicotine addiction varies in adolescent smokers, depending on the type of cigarettes they smoke. In a study [37], 19.4% of the adolescents who smoked every week were considered addicted according to the international classification of diseases (ICD). Even the tobacco consumption less than once a week may progress towards nicotine addiction. It is assumed that the most susceptible young people lose their autonomy on tobacco consumption after 1-2 days since the first inhalation of cigarette smoke. The onset of withdrawal symptoms and the failed attempts to cease smoking may precede tobacco addiction with daily consumption as defined by the ICD-10, and typically occurs before consumption reaches 2 cigarettes a day [36]. In a study using data from the National Survey on Drug Use & Health,a 28% prevalence of nicotine addiction is reported during the previous year in adolescents aged between 12-17 years who smoked during the previous month, this level being only slightly lower
than the prevalence in adults aged between 18-49 years [38].

Colby et al. [33] conducted a meta-analysis observing the experience of some adolescents prior to them starting to smoke. Most adolescents reported at least one withdrawal symptom. The most reported symptom was a strong urge to smoke. Fernando et al. [39] analyzed the data from the National Youth Tobacco Survey and reported that 63% of the adolescents who smoked 5 cigarettes or less a day described at least one withdrawal symptom. Hanson et al. [40] examined the effects of nicotine patch applications on withdrawal symptoms in adolescents. As compared to the placebo group, the group using nicotine patches had lower levels of withdrawal symptoms.

3. Tobacco use for weight control
Numerous adolescents and young adults think that smoking helps weight control, but the proportion in which this practice influences the smoking habit is not known. Nevertheless, there are many studies in which young subjects were questioned regarding the methods used for weight control, as well as the reasons they smoke, in an effort to determine whether young people use smoking as a weight control strategy.

In one study, Klesges et al. [43] interviewed 204 male and female adolescents regarding the strategies they used during the previous 6 months to control their body weight. In addition to the usual methods such as limitation of the energy consumption, skipping meals, eating less and controlling food rations, a number of respondents indicated the use of smoking or caffeine as weight control strategies. As smoking and caffeine intake were combined to a single study element, the authors could not determine the percent of respondents using each method. Generally, women (21%) had significantly more chances than men (4%) to use this combined strategy. The use of smoking/caffeine for weight control was positively associated with body weight, overweight men and women most probably using this method (22%), followed by those with normal body weight (13%) and by underweight subjects (2%).

In a study conducted by French et al. [44] and revised by Potter [45], the association between body weight, diet and smoking initiation was studied in a group of 1705 adolescents. Girls who had more than two symptoms of feeding disorders, those who tried to lose weight during the previous year and those who were concerned with their own body weight had twice as many chances to start smoking during the following year as compared to girls not included in the above classifications. Food restrictions, concerns regarding the possibility of weight gain and the wish to be slim were not associated with smoking initiation. In boys, none of the concerns regarding body weight were associated to the onset of smoking.

Austin and Gortmaker [46] prospectively investigated the association between the frequency of dieting and the initiation of

Killen et al. [41] recruited adolescents from several high schools, but also homeless adolescents who smoked at least 10 cigarettes a day and classified them into two groups, one group who used nicotine patches and the other using placebo patches. The researchers found a decreased cardiac rate only in the placebo group. They found significant increases in the levels of withdrawal symptoms both in the nicotine patch and in the placebo groups. The most important withdrawal symptoms were urge and anxiety which were not alleviated even after the use of nicotine patches.

Prokhorov et al. [42] suggested that nonspecific symptoms such as irritability, depression, insomnia and focus difficulties should be interpreted with caution, as these may have multiple causes other than tobacco withdrawal.
smoking in a group of 1295 adolescents participating in an interventional study on nutrition and physical activity topics. In non-smoking adolescents, the frequency of dieting was a significant predictor of smoking initiation. In the group who did not report during the basal measurements to be on a diet, after applying a multivariate logistic regression model, results showed that girls who only dieted once a week had 1.98 times more chances to start smoking as compared to those not on a diet. For those who reported to be on a diet more than once a week, the chances to initiate smoking were 3.9 times higher than for those not dieting. In boys, the frequency of dieting was not associated to an increased probability to initiate smoking.

Stice and Shaw [47] prospectively examined the relation between the body image and feeding and affective disorders and the initiation of smoking in a group of girls with the median age of 13 years at the time they were included into the study. Between the initial moment and the first year of follow-up, 6% of non-smokers became experimental smokers and 5% became daily smokers. In a multivariate logistic regression model in which negative effects were controlled for, girls with a high level of dissatisfaction regarding their body image and with feeding pathology, were more than four times more susceptible to initiate smoking than those who did not have a high level of discontentment.

Blitstein [48] examined the factors associated to the transition speed through the stages of smoking among adolescents who did not smoke at the beginning of the study. Students who progressed from non-smokers to regular smokers (at least weekly smokers) during one year, were classified as rapidly progressing. Those who progressed from the stage of non-smoker to experimental smokers (less than weekly smokers) were considered slow progressers. The belief that smoking influences body weight was not correlated with the speed of smoking progression neither in boys nor in girls. Nevertheless, girls who reported more serious problems with the diet, had significantly more chances to rapidly progress from non-smokers to regular smokers. In boys, no association was observed between diet and smoking progression.

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Received for publication: 10.01.2012, Revised: 08.03.2012
ASSOCIATION BETWEEN ALCOHOL CONSUMPTION AND SMOKING IN TIMIS COUNTY STUDENTS

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REZUMAT

Studiul a avut ca scop investigarea asocierii între două comportamente de risc pentru sănătate în cazul persoanelor tinere: consumul de alcool și fumatul. Eșantionul reprezentativ de elevi a inclus 2076 de tineri din Timiș, din mediul urban, 62,5% fete și 37,5% băieți, cu vârste cuprinse între 18-25 ani. Metoda de lucru a fost de studiu transversal polulational bazat pe CORT 2004 chestionar vizând comportamentele cu risc la adolescenți și tineri. Prelucrarea și interpretarea datelor utilizate au fost realizate cu programul EpiInfo, versiunea 3.5.1, 2008. Elevii fumători și cei cu o frecvență crescută a fumatului au consumat băuturi alcoolice într-un număr semnificativ mai mare de zile în cursul lunii precedente, au consumat alcool în exces în mai multe zile în cursul lunii precedente și au fost beți mult mai des decât non-fumătorii, diferențele fiind de marime medie, în circumstanțele date. Rezultatele studiului confirmă datele din literatura internațională.

Cuvinte cheie: studenți, consum de alcool, fumat

ABSTRACT

The study aimed to investigate the association between two health risk behaviours in young people: alcohol consumption and smoking. The representative sample of students included 2076 young people in Timiș county universities, urban residents, 62.5% girls and 37.5% boys, aged between 18-25 years. The working method was the transversal polulational study based on the CORT 2004 Questionnaire concerning health risk behaviours in adolescents and young people. Data processing and interpretation used the Epiinfo programme, version 3.5.1, 2008. Smoker students and those with an increased smoking intensity consumed alcoholic beverages on a significantly higher number of days during the previous month, consumed alcohol excessively for more days during the previous month and became drunk significantly more often than non-smoker students, the differences being of medium size under the given circumstances. The results of the study confirm international literature data.

Keywords: students, alcohol consumption, smoking
INTRODUCTION

Researches on risk behaviours in young people correlatively prove the association between alcohol and tobacco consumption. Persons who consume alcohol during social events are more likely to be smokers than those who do not drink and alcoholics are more likely to be smokers than non-alcoholics. Similarly, smokers are more likely to drink alcohol than non-smokers. The co-variation between alcohol and tobacco consumption transcends gender, age and culture [1-3].

Important predictive factors have been identified for both alcohol and tobacco involvement among which: family histories of alcoholism, temper and personality (low behavioural control and negative affectivity), social factors (parental and peer shaping), stress and substance consumption beyond expectations [4].

Since alcohol and tobacco are generally the first drugs experimented by adolescents, it is probable that the initiation of alcohol consumption in non-drinkers to be strongly associated to previous smoking, and smoking initiation in non-smokers to be strongly associated to previous drinking [5].

There is fundament for the hypothesis according to which alcohol consumption has a predictive character for smoking, stronger than in the reversed relation. For both alcohol consumption and smoking, the onset and persistence are mutually anticipated by the use of the other substance, and these associations are robust in gender groups [6].

MATERIAL AND METHOD

The representative sample of Timis County students (the size of the sample stratified in clusters with the aid of the Epiinfo programme, version 6.04, 2001) totaled 2976 young subjects in Timis County universities, with urban residence. The gender distribution was as follows: 62.5% girls and 37.5% boys. The age of the students was between 18-25 years, with the highest proportion of 21 year olds, 27.2%.

The working method was the transversal populational study based upon the use of the CORT 2004 Questionnaire on health risk behaviours in adolescents and young people, performed during the type A CNCSIS grant, code 1167, 2003-2005, entitled: “Assessment of the magnitude of risk behaviours in highschool pupils and young people in undergraduate, postgraduate, occupational and university teaching systems in Timis County”.

The CORT 2004 Questionnaire represents an original working instrument belonging to the research team of the project and it has been developed from other questionnaires used for the investigation of risk behaviours, mainly the European ESPAD and the American YRBSSS. The questionnaire was validated by the Ethics Commission of the “Victor Babeș” University of Medicine and Pharmacy, Timișoara.

Highschool pupils were included into the study only after freely expressed informed consent obtained from each participant. The general response rate was 40%.

Data processing and interpretation used advanced modern methods of medical statistics, with the Epiinfo programme, version 3.5.1, 2008.

RESULTS AND DISCUSSIONS

1. Alcohol consumption during the previous month

Depending on the number of days of the last month in which students consumed at least one portion of alcohol, in the case of non-smokers we obtained a percent of 56.1 (784) who never drank, 35.8% (500) who drank on less than 5 days, 4.5% (63) who drank on 6 to 9 days and 3.1% (43) who drank on more than 10 days. In the group of smoker students, we obtained the following results:
29.5% (190) never drank, 50.6% (325) drank on less than 5 days, 11.2% (72) drank on 6-9 days and 8.1% (52) drank on more than 10 days.

Smoker students consumed at least one unit of alcohol on a significantly higher number of days during the last month as compared to non-smoker students, \( U=307867.5, z=-12.27, p<0.001 \), the difference being of average magnitude (Figure 1).

![Figure 1. Percent distribution of students according to the number of days during the last month when at least one portion of alcohol was consumed correlated to the smoking status](image)

Using only the group of smoker students, we found that smoking intensity is influenced by the number of days in the last month when at least one alcohol portion was consumed, \( H(2)=19.10, p<0.001 \). We applied the Mann-Whitney test to investigate this result. We applied a Bonferroni correction and the effects were reported to a level of significance of 0.0167. We found that students who have an increased smoking intensity drank at least one portion of alcohol on more days during the last month as compared to students who smoke with a medium intensity, \( U=16662.5, z=-3.26, p<0.01 \), and that there are no differences between students who smoke with a medium and light intensity regarding the number of days on which they drank at least one portion of alcohol (Figure 2).
Figure 2. Percent distribution of students according to the number of days during the last month on which they drank at least one portion of alcohol and the intensity of smoking

2. Excessive alcohol consumption during the last month
Depending on the number of days in the last month on which students consumed at least 5 consecutive portions of alcohol during several hours, we found, in non-smokers, 88.5% (1236) who never drank, 9.8% (137) who drank on less than 5 days, 0.6% (8) who drank on 6 to 9 days and 1.1% (15) who drank on more than 10 days. In the group of smoker students, we obtained the following results: 62.4% (402) who never drank, 32.5% (209) who drank on less than 5 days, 3.4% (22) who drank on 6 to 9 days and 1.7% (11) who drank on more than 10 days.

Smoker students consumed at least 5 consecutive portions of alcoholic beverages during several hours, on significantly more days during the last month as compared to non-smoker students, $U=330183.5$, $z=-13.90$, $p<0.001$, the difference being of medium size (Figure 3).
Figure 3. Percent distribution of students according to the number of days on which they drank during the last month and the smoking status

Using only the group of smoker students, we found that the intensity of smoking is influenced by the number of days during the last month on which they drank 5 or more consecutive portions of alcohol during several hours, $H(2)=31.15$, $p<0.001$. We applied the Mann-Whitney test and the Bonferroni correction and the effects were reported to a level of significance of 0.0167. We found that the students with an increased smoking intensity drank 5 or more consecutive alcohol portions during several hours in the last month as compared to students smoking with a medium intensity, $U=16472$, $z=-3.83$, $p<0.01$. There are no differences between students who smoke with medium or light intensity regarding the number of days on which they drank 5 or more consecutive alcohol portions during several hours (Figure 4).
3. The number of drunkenness occasions throughout the entire life
Depending on the number of occasions they got drunk, in the group of non-smoker students we found 55.4% who never got drunk, 31.1% (435) who got drunk less than 5 times, 8.9% (124) who got drunk between 6 and 19 times and 4.7% (65) who got drunk more than 20 times. In the group of smoker students the distribution is as follows: 19.0% (121) never got drunk, 40.4% (258) got drunk less than 5 times, 21.2% (135) got drunk between 6 and 19 times, and 19.4% (124) got drunk more than 20 times.

Smoker students got drunk significantly more often as compared to non-smoker students, U=241804, z=-17.50, p<0.001, the difference being of medium size (Figure 5).
Using only the group of smoker students, we found that the intensity of smoking is influenced by the number of times they got drunk, $H(2)=31.20$, $p<0.001$. The Mann-Whitney test and Bonferroni correction were applied and the effects were reported to a significance level of 0.0167. We found that students with an increased smoking intensity got drunk more frequently as compared to those who smoke with a medium intensity, $U=16376$, $z=-3.21$, $p<0.01$. There are no differences between students who smoke with medium or light intensity regarding the number of occasions they got drunk (Figure 6).
A study conducted in Great Britain [7] indicated a strong association between smoking and alcohol consumption, and the chances to be a daily smoker increase with the number of alcohol units consumed during the previous week. Grant [8] found that the early onset of smoking is associated to early alcohol consumption, as well as with the risk to further develop alcohol related disorders. A transversal study conducted by Koopmans et al. [9], found that adolescents and young adults who smoked had higher chances to consume alcohol as compared to their non-smoker peers, and the relation seemed to be environmentally rather than genetically mediated.

Other authors [10] found a positive association between the incidence of alcohol related disorders and nicotine addiction. More recently, Weitzman and Chen[11] found that 98% of the students who smoked, also consumed alcohol, and 59% of students who consumed alcohol were also smokers. The risk of double consumption was the highest in students with the highest alcohol consumption level.

**CONCLUSIONS**

Depending on the number of days in the last month on which students consumed at least one portion of alcohol, in non-smokers / smokers we found the following: 35.8% / 50.6% drank on less than 5 days, 4.5% / 11.2% drank on 6 to 9 days; 3.1% / 8.1% drank on more than 10 days. Smoker students consumed alcoholic beverages on significantly more days during the last month as compared to non-smoker students, the difference being of medium size. Students smoking with an increased intensity drank at least one portion of alcohol on several days during the last
month as compared to those who smoke with medium intensity.

Depending on the number of days on which they consumed at least 5 consecutive portions of alcohol during several hours, the situation in smokers / non-smokers is the following: 9.8% / 32.5% drank on less than 5 days; 0.6% / 3.4% drank on 6 to 9 days; 1.1% / 1.7% drank on more than 10 days. Smoker students consumed alcoholic beverages in excess on significantly more days during the last month as compared to non-smoker students, with a medium size difference between the two groups. Students smoking with high intensity drank on significantly more days 5 or more consecutive alcohol portions in several hours during the last month as compared to medium intensity smoker students.

Depending on the number of occasions on which smokers and non-smokers got drunk we found the following situation: 31.1% / 40.4% got drunk less than 5 times; 8.9% / 21.2% got drunk between 6 and 19 times; 4.7% / 19.4% got drunk over 20 times. Smoker students got drunk significantly more often as compared to non-smoker students, the difference being of medium size. Students who smoke with high intensity got drunk more frequently as compared to medium intensity smoker students.

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Received for publication: 10.01.2012, Revised: 28.03.2012
A COMPARISON OF TWO METHODS FOR PREDICTING THE SIZE OF UNERUPTED PERMANENT CANINES AND PREMOLARS. NEW REGRESSION EQUATIONS OF PREDICTING THE SIZE OF UNERUPTED CANINES AND PREMOLARS

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ABSTRACT

The aim of this study was to evaluate the applicability of the methods Moyers (1988) and Tanaka and Johnston (1974) for predicting the mesio-distal diameter of canine and premolars and to propose a new regression equations. The sample consisted of 52 (33 female and 19 male, sex ratio 1.73:1 F/B) Romanian patients with mixed dentition treated in the Orthodontic and Dentofacial Clinic, at the Faculty of Dentistry, University of Medicine and Pharmacy “Carol Davila”, Bucharest. Measurements of teeth were made with a modified digital caliper. The Moyers (1988) and Tanaka and Johnston (1974) were tested and the results were compared with the mesiodistal diameter of canine and premolars after their eruption.
erupting. Three linear regression equations were determined to estimate the widths of unerupted lower canine and premolars, for whole sample and each sex.

**Keywords:** mixed dentition, prediction, premolars, regression equation

**INTRODUCTION**

The ability of predicting the size of unerupted teeth in mixed dentition is an important stage to set a right treatment plan. Space analysis of mixed dentition helps to establish if the orthodontic treatment involve serial extractions, space maintenance, guidance of eruption or periodic observation of the patient [1-4] and shows the space available in the posterior segment is sufficient to allow the permanent teeth to erupt well-aligned [5].

Three main approaches were described for tooth size prediction of unerupted canines, first and second premolars:
1. measurement of the unerupted teeth on the radiographs (Nance 1947 [6]; Bull 1959; Huckaba 1964; De Paula and al. 45º oblique teleradiographs [7];

Tanaka and Johnston (1974) is the most commonly used method in predicting unerupted tooth size, because of its acceptable accuracy for both jaws and both genders in general [10-13].

**AIM AND OBJECTIVES**

The aim of the study was to evaluate the applicability of the Tanaka and Johnston (1974) an Moyers (1988) methods of predicting the size of the unerupted lowers permanent canines, first and second premolars.

First objective was to calculate the mesio-distal diameters of the unerupted lower permanent canines and premolars from Tanaka and Johnston (1974) equation and the Moyers prediction tables (Moyers 1988).

Second objective was to compare the predicted values of mesio-distal diameters of permanent lower canine and premolars from Tanaka and Johnston (1974) with those from Moyers (1988) method.

Third objective was to compare the predicted values of mesio-distal diameters of the permanent lowers canine and premolars from each of these methods with the measurement values after teeth eruption.

The last objective was to propose a new regression equations.

**MATERIALS AND METHODS**

Fifty two dental study casts and 52 panoramic radiographs were selected from patients with mixed dentition attending Orthodontic and Dentofacial Cline at Faculty of Dentistry, University of Medicine and Pharmacy “Carol Davila”, Bucharest. The age of the 52 patients (33 female patients and 19 male patients, sex ratio 1.73:1 F/B) range from 7.9 to 10.2 years old.

The criteria of selection were: all the patients with mixed dentition; teeth without approximal decay, fractures or any mesial or distal restorations; all lower incisors should be present or fully erupted; without extraction; no hypodontic; high quality study cast without any damage; panoramic radiographs for diagnosis any hypodontia, a specially in lateral area; study casts from the beginning of the study (with mixed dentition) and from the final (with all teeth fully erupted).

The measurements of erupted teeth can be made in oral cavity or on the dental cast. In the present study the measurement were
made on the dental casts with a modified digital caliper. The electronic digital caliper Hedü (150 mm) had an accuracy of +/- 0.1mm and resolution 0.01mm. On the initial study casts were measured all the mesio-distal width of lower central and lateral incisors between of two anatomical points of each tooth parallel with the incisal edge of the tooth and also parallel with the vestibular surface of the model. For a high accuracy the digital caliper was modified with two wipla rods (0.8 mm diameter and 5 cm length)(Figure 1).

In the last part of the study were made measurements of the lowers canine, first and second premolars erupted and were compared with values calculated from Tanaka and Johnston (1974) and Moyers (1988) methods; the gender distribution of the values was made. Descriptive statistics including means, standard deviations, minimum and maxim values, were calculated with PASW 18 (SPSS); paired t-test were used to compare right-left values and predicted values with those measured; a probability p>0.05 was taken as not significant; p<0.05 as significant; p<0.001 as very highly significant.

RESULTS

The sum of lower incisors were use to predict the combined size of unerupted canines, first and second premolars using both Moyers (1988) and Tanaka and Johnston (1974).

Tanaka and Johnston (1974) method results:
For applying Tanaka and Johnston method (1974) was used the equation:
\[
\sum_{C,Pm1 \text{ si } Pm2} = \sum_{II} \times 0.5 + 10.5
\]
\[
\sum_{II} = \text{sum of incisors}
\]

Minimum and maximum values of lower canine and premolars calculated are presented below (Figure 2 and Table 1):
The sum of lower canine and premolars calculated by Tanaka and Johnston method (1974) was found significant higher for male sample than female sample p<0.05.

Moyers(1988) method results (Figure3)Moyers method use prediction tables for calculated the sum of canine and premolars, with probability level 5%-95%.
Figure 3. Minimum and maximum values for calculated sum of lower canine and premolars by Moyers method(1988) at 5%-95% probability level. Measurements are in mm

For the study sample the values range from 17.8mm for the level of probability 5% up to 23.2mm for the level of probability 95%. At 75% the sum of lower canine and premolars was 20.6-22.1 mm, mean value for female 21.36 mm and 22.32 mm for male sample.

Measurements values after teeth eruption:
Sum of mandibular canine and premolars was found greater in male sample than female sample on the third and fourth quadrant ; the mean difference between gender was found 1.17 mm on the left side and 1.157mm on the right side. No statistically difference was found between left or right side for whole sample t(51)=-0.991, p>0.05. Mean difference left-right side was -0.028 and standard deviation 0.2099 (Table 2).

Table 2. Values of canine and premolars sum measured after teeth eruption for whole sample. Measurements in mm

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actual value for third quadrant</td>
<td>22.0</td>
<td>20.9</td>
<td>23.5</td>
</tr>
<tr>
<td>Actual value for fourth quadrant</td>
<td>22.1</td>
<td>21</td>
<td>23.5</td>
</tr>
</tbody>
</table>
Comparisons of predicted values from Tanaka and Johnston method (1974) with actual tooth size

The predicted sum of canine and premolars from Tanaka and Johnston (1974) method was significant higher than actual sum of canine and premolars $t(51)= 2.03, p<0.05 \ r=0.27$, low correlation; the mean difference was 0.0961mm. For female sample the predicted values were significant higher than the actual sum, $t(32)= 30, p<0.001, r=0.98$, high correlation; mean difference between the two values was 0.349 mm. For the male sample, the actual sum of canine and premolars is significant higher than the predicted values, $t(18)= -13.02, p<0.001, r=0.95$, high correlation.

Comparisons of predicted values from Moyers method (1988)-probability level 5%-95% with actual tooth size

Statistically, we found the actual sum of canine and premolars was significant higher than calculated sum from Moyers (1988) method; at the probability level 85%, the difference was not found significant $p>0.05$; and at 95% the actual sum was significant lower than those predicted $t(51)=39.79, p<0.001, r=0.98$, high correlation.

For female group the actual sum is significant higher than Moyers (1988) at probability level 5%-75%. At 85% and 95% actual sum is significant lower than predicted values $t(32)=6.34, p<0.001, r=0.98$, high correlation respectively $t(32)=44.37, p<0.001, r=0.98$, high correlation.

For male sample the actual values are significant higher than predicted at probability level 5%-85% and at 95% actual sum is significant lower than those predicted $t(18)=33.41, p<0.001, r=0.98$, high correlation (Table 3 and 4).

Table 3. Comparison of predicted values from Tanaka and Johnston (1974) and Moyers (1988) methods with actual values for male sample. Measurements made in mm

<table>
<thead>
<tr>
<th>Male patients</th>
<th>Predicted values $\sum C, Pm1$ and $Pm2$</th>
<th>Actual values $\sum C, Pm1$ and $Pm2$</th>
<th>Mean difference</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Std. Deviation</td>
<td>Mean</td>
<td>Std. Deviation</td>
</tr>
<tr>
<td>Moyers 50% (1988)</td>
<td>21.52</td>
<td>0.479</td>
<td>22.837</td>
<td>0.466</td>
</tr>
<tr>
<td>Moyers 75% (1988)</td>
<td>22.32</td>
<td>0.479</td>
<td>22.837</td>
<td>0.466</td>
</tr>
<tr>
<td>Tanaka and Johnston (1974)</td>
<td>22.50</td>
<td>0.538</td>
<td>22.837</td>
<td>0.466</td>
</tr>
</tbody>
</table>
Table 4. Comparison of predicted values from Tanaka and Johnston (1974) and Moyers (1988) methods with actual values for female sample. Measurements made in mm

<table>
<thead>
<tr>
<th>Female patients</th>
<th>Predicted values $\Sigma C, Pm_1$ and $Pm_2$</th>
<th>Actual values $\Sigma C, Pm_1$ and $Pm_2$</th>
<th>Mean difference</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Std. Deviation</td>
<td>Mean</td>
<td>Std. Deviation</td>
</tr>
<tr>
<td>Moyers 50% (1988)</td>
<td>20.56</td>
<td>0.437</td>
<td>21.66</td>
<td>0.408</td>
</tr>
<tr>
<td>Moyers 75% (1988)</td>
<td>21.36</td>
<td>0.437</td>
<td>21.66</td>
<td>0.408</td>
</tr>
<tr>
<td>Tanaka and Johnston (1974)</td>
<td>22</td>
<td>0.404</td>
<td>21.66</td>
<td>0.408</td>
</tr>
</tbody>
</table>

Regression equation for the study sample

The regression equations for the whole sample and each sex are described below:

- Y = dependent variable (sum of canine, first and second premolars)
- Y = $0.6221x + 7.5495$

$R^2 = 0.8032$

Regression equation for the whole sample, Figure 4

Y = $10.202 + 0.498x$ (prediction equation for female sample, Figure 5)
Figure 5. Graphic comparision of predicted values from Tanaka and Johnston (1974) and Moyers (1988) at 50%-95% probability levels and prediction equation for female sample

\[ y = 0.498x + 10.202 \]
\[ R^2 = 0.974 \]

Figure 6. Graphic comparision of predicted values from Tanaka and Johnston (1974) and Moyers (1988) at 50%-95% probability levels and prediction equation for male sample

\[ y = 0.4269x + 12.589 \]
\[ R^2 = 0.9711 \]

**DISCUSSION**

Bilateral symmetry. It found no significant difference for the sum of canines, first and second premolars, between the third quadrant and the fourth, \( t(51) = -0.991 \), \( p > 0.05 \); mean difference (IV-III) 0.028 mm.

Sex differences. The sum of lower incisors is significant higher for male group than the female, \( t(51) = -3.78 \), \( p < 0.001 \), \( r = 0.47 \), high correlation, mean difference: 0.99 mm. Also, the lower sum of canines, first and second premolars are greater for male sample than the female in both quadrant.

The results of this study shows the limitations of Tanaka and Johnston (1974) and Moyers (1988) methods in predicting the width of unerupted tooth. While some
researchers found an overestimation of the size of the canines, first and second premolars, when using Tanaka and Johnston (1974) method [14,15,16], other reported underestimation [17].

It found no significant difference between the predicted sum of canines and premolars for Tanaka and Johnston (1974) method and the actual values, the mean difference is 0.0961mm, for the whole sample. For female sample the predicted size was significant higher than actual size (mean difference: 0.349mm); for male group the predicted sum was smaller than actual sum (mean difference: 0.334mm). This shows that Tanaka and Johnston (1974) method is suitable for patients in general, but not for each sex.

Although it is used more often, Moyers (1988) predicted values are lower than actual values. The actual sum of canine and premolars (mean value: 22.09mm) are higher than those predicted by Moyers (1988) method at 5%, 15%, 25%, 35%, 50%, 65% and 75% probability level, the mean difference range from 0.376 mm to 3.113mm. No significant difference it was found at 85% probability level and at 95% the actual values are smaller than those predicted. For female group the predicted values at predictability level 5%, 15%, 25%, 35%, 50%, 65% si 75% are smaller than actual values (mean value: 21.66mm) and at 85%, 95% they are higher. For male sample the predicted sum of canines and premolars to a probability level 5%, 15%, 25%, 35%, 50%, 65% si 75% si 85% are smaller than actual sum, at 85% it’s not significant and at 95% are higher; the mean value for the actual sum of canines and premolars is 22.83 mm (for the male group).

**CONCLUSIONS**

1. Moyers (1988) prediction tables are not suitable for estimating the combined mesio-distal widths of unerupted of the canines and two premolars. The values are underestimated.
2. Tanaka and Johnston (1974) seems to be suitable for clinical use in the mixed dentition analysis for whole patients in general, but not suitable for each sex.
3. New equation was developed to allow clinicians to accurately predict the diameter of unerupted canines and premolars.

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Received for publication: 09.01.2012, Revised: 22.03.2012
EVALUATION OF THE ANNOYANCE PROVOKED TO PERSONS BY THE ENVIRONMENTAL NOISE IN TIMISOARA

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2. Ministry of Environment, IB SOP Environment Timisoara
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REZUMAT


Cuvinte cheie: zgomot ambiental, disconfort

ABSTRACT

Environmental noise has become a global problem and represents one of the major public health risks. Exposure to noise is increasing over the time, compared to other environmental factors. We have proposed an evaluation of the annoyance produced by the environmental noise upon the study of the complaints addressed by the citizens to the environmental protection authorities in Timisoara. This study covers four years, 2008-2001, and has revealed that the annoyance by noise is induced during the night by the activities in the commercial and entertainment zones (bars, clubs, restaurants, discos) and during the day, by the activities of the small businesses located in the residential areas.

Keywords: environmental noise, annoyance
INTRODUCTION
The environmental noise has become a common and widespread problem. Although legislative measures were taken, action plans designed and noise mapping implemented, there are little significant results.

This may be because even at low acoustic levels noise can induce sleep disturbance, learning and working problems and emotional issues. That’s why noise is also known as „unwanted sound” [1].

The concept of unwanted sound is closely related with annoyance. Studies have shown that the level of annoyance rises with the increase of the acoustic pressure levels [2].

However, at lower noise levels, other factors decide if annoyance is present or not [3].

In some European countries, a special noise indicator is used: the rating level, Lr. Lr is based on the continuous equivalent noise level, LAeq, upon which penalties are applied. These penalties are added for impulsive noise, tonal noise, time of day and type of noise source. Although widely used, in Germany for example, Lr has some major disadvantages: a weak correlation between the noise level and annoyance, and the impossibility to offer details about the reasons of the annoyance.

LAeq could be a good annoyance predictor [4, 5], if it comes together with supplemental indicators and an expert’s opinion related to the studied case. The expert’s opinion could take into account various other data, like the ones emerging from a questionnaire applied to the noise affected population.

Noise affects differentiately people. Many times a high acoustic pressure level does not cause annoyance if it occurs in acceptable circumstances, if it origins from controllable sources or if the individual’s psychological status is a tolerant one. Because a number of studies show the difficulties in the correlation of the acoustic indicators and the level of annoyance [1, 6-8] for a population, it would seem better to asses the individual annoyance for a person exposed to environmental noise.

In this paper, a evaluation of annoyance generated by environmental noise was made for the citizens of Timisoara by the means of case studies.

MATERIAL AND METHOD
For the identification of the urban sources of noise, generators of annoyance, and for the assessment of the annoyance level induced by noise, a research of the written complaints of the citizens of Timisoara was conducted.

These complaints, referring to a wide range of environmental issues, were addressed by the citizens, via regular mail or e-mail, to the environmental protection authorities of Timisoara, the National Environmental Guard and the Environmental Protection Agency.

A detailed study of the complaints archive of the last four years (2008-2011) was conducted in order to asses the percentage of noise related complaints, and the distribution of the noise sources that generated the annoyance that led to the writing of complaints.

For the year 2008, 324 complaints were reviewed, for 2009, 407 complaints, for 2010, 309 complaints and for the year 2011, 285 complaints, totaling 1,325 complaints.

RESULTS AND DISCUSSION
The study of the National Environmental Guard Timisoara and Environmental Protection Agency Timisoara archives revealed, for the four year period 2008-2011, the following aspects.
Form the total of the written complaints, 33% were related to environmental noise issues (Figure 1).

The majority of the noise complaints (45%) are referring to the night-time entertainment in the commercial zones neighboring the residential area. In approximately equal shares, bars, pubs, restaurants, clubs and discos are held responsible for the high levels of music regarded as annoying noise by the citizens.

![Figure 1. Percentage of complaints regarding the main environmental factors](image1)

![Figure 2. Distribution by source of the noise related complaints](image2)
During the day-time, the main noise generators are the small businesses located within the residential area. During the last 20 years, the old large factories in Timisoara have been closed, diminishing the industrial noise in the central part of the city. In compensation, the number of small businesses rose exponentially, occupying buildings within or in the vicinity of the residential area. The activities performed by wood works, metal works, bakeries, mills, carwash facilities, car service stations, led to a large number of complaints, 36% from the noise related complaints (Figure 2).

Sawing, hammering, loading and unloading of trucks, rumbling of various machines, hissing form air-vents, humming form compressors and coolers are the main sources that generate the most annoyance to the residents.

Cooling stations, ventilators and air conditioning units are responsible for 12% of the complaints. Their constant low frequency sounds generate annoyance of the neighbors especially during night-time, in the summer, when the residual level of noise is lower and residents keep their windows mostly open.

Construction yards count only 2% from the total of the complaints. Mostly these complaints result as a argument between neighbors. A very small number of complaints refer to the annoyance provoked by large infrastructure works such as replacing gas, sewage, and water pipes and then noise is a secondary environmental factor brought to attention, the main ones being air and soil pollution with dust and debris.

Miscellaneous complaints are about 4% from the total. These complaints are a proof that sometimes a noise doesn’t need to be loud to annoy severely a person. In this section, the people complain about various sources, including barking dogs, the noise made by tennis balls in a tennis club field, the buzzing of a street electric transformer or even the loudspeakers used by a church for religious service amplification reasons.

Although road traffic is the main source of environmental noise only a couple of complaints mention it, in connection with poor air quality. A particular case is the traffic of the trams, old vehicles in a rather poor technical condition, that emit noise levels 15-20 dBA higher than average street traffic levels, noise accompanied usually by vibrations. These complaints summed 1%.

A single complaint referred to the noise generated by trains and no complaint was recorded for noise generated by air traffic, although the flights of helicopters and small aircraft have risen in the last decade.

**CONCLUSIONS**

Environmental noise has a significant impact upon the residents of Timisoara. This fact is taken from the large amount of the complaints addressed to the environmental authorities in the last four years (2008-2011), of which noise related complaints represent 33%.

The main noise-induced annoyance factors are the music and noisy customers of the bars, restaurants, clubs and discos, in the night-time, and the activities of the various small businesses like woodworks, metal works, warehouses, carwashes, in the daytime, in the residential area.

The number of complaints related to the main cause of environmental noise – road traffic is very small because it seems that even if road noise annoys, the low controllability factor of the source tends to discourage the citizens to make complaints.
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Received for publication: 14.02.2012, Revised: 29.03.2012
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A number of 3-5 key-words will be given.

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Technical information. Identify the methods, equipments and procedures offering sufficient details to allow other researchers to reproduce the results. Cite reference sources for the used methods by arabic figures between square brackets. Describe new or substantially changed methods, indicating the reasons for using them and assessing their limitations.
Statistics. Describe statistical methods using sufficient details for an informed reader who has access to original data to be able to verify the presented results. Whenever possible, quantify the results and present them accompanied by appropriated indicators for the error or uncertainty of measurement. Specify the used programme for statistical analysis.

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Present the obtained results with a logical sequence in the text, with tables and figures. Do not repeat in the text all data presented in tables and figures; only stress upon and synthesize important observations. Additional materials and technical details may be placed in an appendix where they may be accessed without interrupting the fluidity of the text. Use figures not only as relative (percent) values but also as absolute values from which relative ones have been calculated. Restrict only to necessary tables and figures. Use graphs as an alternative to tables with numerous data. Do not present the same data twice in tables and graphs.

DISCUSSIONS
Stress upon new and important aspects of the study. Do not repeat detailed data from previous sections. Establish the limitations of the study and analyze the implications of the discovered aspects for future research.

CONCLUSIONS
State the conclusions which emerge from the study. Show the connection between the conclusions and the aims of the study. Avoid unqualified statements and conclusions which are not adequately supported by the presented data. You may issue new hypothesis whenever justified but clearly describe them as such.

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Generate tables in Word.
Number tables with arabic figures, consecutively, according to the first citation and give them short titles (Table 1……...); number and title situated at the upper margin and outside the table.
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Insert tables in the text.
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Create black and white graphs, editable in Excel or Microsoft Word.
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