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Development of bacterial resistance in Central Military Hospital Ružomberok, Slovakia

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The purpose of this study was to analyze the resistance during the period of 1998–2002 based on results of quantitative sensitivity (MIC) of selected bacterial pathogens in the Central Military Hospital in Ružomberok, Slovakia (CMH). Analysis of gram-negative bacteria showed that resistance has increased in general. Marked changes were observed especially in values obtained from different departments. The increased cephalosporin consumption is considered to be one of the main driving forces of resistance increase, which also results in increase of sequence pressure of antibiotics and increase in multi-resistant strains in the environment. This fact is, of course, reflected in the consumption of more expensive wide-spectrum and reserve antibiotics. The situation in the hospital just confirms the general trend of increasing the resistance and the need to solve this problem under specific conditions in particular health care facility.

Key words: resistance, gram-negative bacteria, antibiotics pressure, multi-resistant strains.

Abbreviations: Central Military Hospital in Ružomberok (Slovakia), CMH; intensive care unit, ICU; University Hospital in Olomouc (Czech Republic), UHO.

Introduction

Until recently the medical community world-wide has seemed incapable of reacting to the imminent crisis of antibiotic resistance. Many authors in their publications have analyzed the causes of the high and still increasing antibiotic resistance. They stress the meaning of monitoring and suggest different ideas for solving this problem (Tattam, 1998; Handberger et al., 1999; Flournoy et al., 2000; Ibrahim et al., 2000).

Current knowledge and information on mechanism, incidence and spread of bacterial resistance show that overuse of antibiotics principally changes the etiology of pathogenic and facultative pathogenic bacteria. This fact has found its reflection in the creation of stable reservoirs of strains resistant to antibiotics, while genetic structures of antibiotic resistance (plasmids, transposones, intergrones) are able of maintaining in bacterial cells even though selective pressure of antibiotics is absent. Subsequent implementation of various mechanisms of gene transfer between gram-negative

and gram-positive bacteria causes rapid and explosive growth of resistance in previously sensitive strains to new, very effective antibiotics (BLAHOVA et al.,1998).

Surveillance of bacterial resistance is a key element in understanding the size of the problem. The large number of existing networks for resistance surveillance needs to be coordinated and the results have to become available (COHEN, 1997; SAHM & TENOVER, 1997; RAVEH et al., 2001; FELMINGHAM, 2002). Good quality local data provide a basis for national and international surveillance and are necessary to help doctors to choose appropriate antibiotics and to detect local epidemics of resistant bacteria surveillance at a local level (LIVERMORE et al, 1998; MASTERTON, 2000).

Legitimacy of monitoring the antibiotic resistance of microbes is also shown in recently published results obtained in large network of Intensive Care Units throughout Europe, as part of MYSTIC and SENTRY projects (DOERN et al., 1997; PFALLER et al., 1998; TURNER, 2000; HOBAN et al., 2001; GARCIA-RODRIGUEZ & JONES, 2002).

Multiple studies describe incorrect usage of antibiotics (KOLLEF et al., 1999). The main reason is incorrectly founded and excessive usage of wide-spectrum antibiotics, especially in hospital environment. Selective pressure of these antibiotics allows new types of multi-resistant bacteria survive easily (HUYCKE et al., 1998).

There are two ways of how to control the development and spread of resistant bacteria. The first way is to reduce the use of antimicrobial agents in order to decrease selection of resistant bacteria (SCHWARTZ et al., 1997). Restriction policies, such as the requirement for written justification or automatic stop orders may be useful in hospital settings; this has in some cases real justification. To reduce the antibiotic consumption, a multifaceted approach including the education of doctors is necessary; widely accepted recommendations for good clinical diagnosis and treatment (NATHWANI & DAVEY, 1999).

The second major way to tackle the resistance is improving the hygienic measures to prevent the spread of resistant bacteria. The spread of infectious organisms from patient to patient is a big problem. This usually happens through nurses, doctors and other persons who take care of patients. The most common means of spreading these organisms are contaminated hands (DOEBBELING et al., 1992).

The consequences of inappropriate initial therapy are serious and may cause increased mortality and lengthening of stay in intensive care unit (ICU) (DUPONT et al., 2001).

In antibiotic therapy, the empiric treatment generally dominates. The doctor tries to estimate the agent of the disease and agent's sensitivity to antibiotics. Since the national or hospital overviews of sensitivity may be misleading, the doctor needs to rely on representative overview of resistance from particular environment (O'BRIEN, 1997).

The importance of antibiotic resistance control is well-known in the Central Military Hospital in Ružomberok, Slovakia (CMH), too. CMH takes an important position in the wide web of military health care facilities. It is the largest military hospital in the territory of Slovakia. This hospital belongs to the group III.A. type hospitals. The founder is the Ministry of Defense of the Slovak Republic. CMH, although it belongs to the military facilities, offers its services to all citizens of Slovakia, who needs the health care regardless the nationality, occupation or sex.

In this study we compared the sensitivity analysis results obtained from patients at CMH during the past five years. We also analyzed the state of resistance. Considering that ICU gram-negative pathogens are one of the main problematic bacterial groups of strains (SPENCER, 1996), we focused on species of the family *Enterobacteriaceae*, *Pseudomonas* spp. and *Acinetobacter* spp. Since the resistance to antimicrobial substances is a growing problem, especially at ICUs, we were further interested in whether or not there are differences in observed values for the hospital as a whole and values obtained from the ICUs. Finally we focused on analysis of presence of multi-resistant strains and compared the consumption of two groups of antibiotics within last three years.

Material and methods

Gram-negative bacterial strains were isolated from clinical materials (sputum, pus, blood, liquor, urine, bile, exsudates, swabs from the upper respiratory tract and vagina) of patients hospitalized in clinical departments of the CMH.

The bacterial strains were identified to species (genus) level using standard laboratory methods for routine. Exact definitive identification of strains of family *Enterobacteriaceae* was made using the ENTEROtest 16, 24 and Nefermtest (PLIVA – Lachema, Brno, Czech Republic). Configuration of tests enables a highly reliable identification without using other additional tests.

The susceptibility of important strains of *Enterobacteriaceae* was determined by the MIDITECH

automated colorimetric MIC (minimal inhibitory concentration) – reading for antimicrobial susceptibility testing (Bratislava, Slovak Republic). This system is a modification of the standard broth microdilution method that uses a 3-(4,5-dimetylthia007Aol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) dye for detecting viable bacteria (GATTRINGER et al., 2002).

Program package MIDITECH ANALYSER/ MIDISTAT, version 01/03 and double-disk synergy test (JARLIER et al., 1988) were used for determining the resistance mechanisms (method of phenotype analysis) as well as for calculation of statistical sensitivity parameters for particular antibiotics.

Repetition filter in database for isolated strain for particular patient was set for 10 days. The isolates expressing simultaneous resistance to at least three unrelated antibiotics were identified as "multiresistant". We used reference strains *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 35218 a *Pseudomonas aeruginosa* ATCC 27853 for quality control purposes.

We used a standard methodology for calculating the drug consumption. As recommended by WHO (WHO Collaborating Centre for Drug Statistics Methodology: Guidelines for ATC classification and DDD assignment, Oslo, 2002), ABC Calc reports hospital antibiotic consumption as a number of Defined Daily Doses (DDD) per 100 bed-days. DDDs are assigned by the WHO Collaborating Centre for Drug Statistics Methodology (Anatomical Therapeutic Chemical classification index with Defined Daily Doses, Oslo, Norway, 2003) and updated once a year.

Results and discussion

During the period 1980–2002, 3920 gram-negative strains were isolated in total. The most common sites of isolation were urine (36%), wounds (25%), catheter and drain (6%), others (30%). An overview of most common gram-negative sticks causing the bacterial infections in patients hospitalized in CMH during the above-mentioned period can be seen in Table 1.

Table 1. Number of gram–negative bacterial pathogens from CMH.

Pathogen	1998	1999	2000	2001	2002
E. coli	187	189	257	218	322
Pseudomonas spp.	185	232	186	165	183
Proteus spp.	130	89	143	117	151
Enterobacter spp.	63	94	83	92	121
Klebsiella spp.	43	36	71	99	83
Serratia spp.	16	36	37	36	17
Acinetobacter spp.	13	43	25	34	20
Citrobacter spp.	6	18	21	26	33
Total	643	737	823	787	930

The total number of eight most common pathogens, at which we set the quantitative sensitivity by using MIC, increased from 1998 to 2002 by 45%. When comparing their percentage share on the total number, we found the greatest increase in *Klebsiella* spp., *Enterobacter* spp. and *Escherichia coli.* Other strains increased their occurrence modestly. The most commonly identified strains were *E. coli*, *Pseudomonas* spp., *Proteus* spp, *Enterobacter* spp. and *Klebsiella* spp.

Observed strains acquired at ICU of CMH in 2002 represented only 9% of the total 930 bacterial strains (Table 2). Out of 84 strains analyzed in 2002, *Pseudomonas* spp. had the biggest share (44%), followed by *Enterobacter* spp. (18%) and *Klebsiella* spp. (15%); percentage representation of other strains was below 10%.

The majority of isolations at this department were from urine 25%; 24% from catheter and drains, 21% from sputum, 3% from wounds and 27% from other materials.

Table 2. Number of gram-negative bacterial pathogens from ICU of CMH.

Pathogen	1998	1999	2000	2001	2002
Citrobacter spp.	–	–	–	–	5
Enterobacter spp.	6	25	11	14	15
E. coli	15	29	7	10	7
Klebsiella spp.	14	6	11	16	13
Proteus spp.	16	11	15	18	7
Serratia spp.	–	6	11	14	–
Acinetobacter spp.	6	26	12	17	–
Pseudomonas spp.	49	47	58	40	37
Total	106 (17%)	150 (20%)	125 (15%)	129 (16%)	84 (9%)

Table 3. Comparison of prevalence rates obtained by the ICUs of CMH and UHO and surveillance programs MYSTIC and SENTRY monitoring ICU infections (arranged).

Pathogen	ICU CMH 1998–2002	ICU UH O 2001	MYSTIC 1997–2000	SENTRY 2001
Citrobacter spp.	5 (1%)[a]	–	–	–
Enterobacter spp.	71 (12%)	28 (13%)	699 (13%)	92 (15%)
E. coli	68 (11%)	35 (16%)	1237 (23%)	134 (22%)
Klebsiella spp.	60 (10%)	43 (20%)	870 (17%)	117 (20%)
Proteus spp.	67 (11%)	4 (1%)	313 (6%)	–
Serratia spp.	31 (5%)	12 (5%)	321 (6%)	40 (7%)
Acinetobacter spp.	61 (10%)	12 (5%)	426 (8%)	53 (9%)
Pseudomonas spp.	231 (39%)	83 (38%)	1404 (27%)	161 (27%)
Total	594	217	5270	597

[a]Number of isolates (% of total).

Table 4. Resistance of microorganisms to selected antibiotics (in %).[a]

Microorganism	AMP	A+IB	PIP	P+IB	CXM	CTX	CAZ	CPO	MEM
Citrobacter spp.	83/100	50/79	20/45	0/3	67/88	20/36	20/42	0/33	0/3
Enterobacter spp.	100/100	100/100	27/27	9/9	94/100	17/26	18/25	13/19	0/0
E. coli	57/41	27/7	38/20	5/0	14/8	9/5	8/4	9/5	1/0
Klebsiella spp.	100/100	40/16	61/36	15/5	28/35	27/27	27/28	26/24	6/0
Proteus spp.	64/65	50/41	25/26	11/1	51/42	7/3	9/4	9/2	3/0
Serratia spp.	94/82	67/71	67/18	20/18	88/73	43/20	20/0	13/12	0/0
Acinetobacter spp.	100/100	16/20	100/100	36/40	92/100	37/45	27/21	9/25	0/15
Pseudomonas spp.	100/100	100/100	36/21	26/11	100/100	100/100	26/12	8/8	2/3

Microorganism	GEN	AMI	CIP	COT
Citrobacter spp.	0/21	0/0	25/48	0/31
Enterobacter spp.	11/18	2/1	9/14	8/18
E. coli	6/7	2/0	7/15	19/15
Klebsiella spp.	26/26	12/1	13/26	23/20
Proteus spp.	22/32	5/2	20/37	36/40
Serratia spp.	38/24	0/0	14/29	75/18
Acinetobacter spp.	67/35	18/15	86/45	23/50
Pseudomonas spp.	38/40	17/15	42/51	100/100

[a]Comparison between the years 1998 and 2002. The abbreviations: AMP, ampicillin; A+IB, ampicillin+sulbactam; PIP, piperacillin; P+IB, piperacillin+sulbactam; CXM, cefuroxime; CTX, cefotaxime; CAZ, ceftazidime; CPO, cefpirome; MEM, meropenem; GEN, gentamycin; AMI, amikacin; CIP, ciprofloxacin; COT, trimetoprim+sulfonamide.

Comparison of adequate frequency of occurrence of observed types (of strains) at ICU of CMH during 1998–2002 with frequency of occurrence of these types of strains in MYSTIC Program (Garcia-Rodriguez & Jones, 2002), in SENTRY Program (Jones, 2003), and at (ICU) of the University Hospital, Olomouc, Czech Republic (UHO) (Kolář et al., 2002) can be found in Table 3. The prevalence rate of *Pseudomonas* spp. observed at ICU of CMH was 80–90% higher than the European average of G-pathogens in MYSTIC and SENTRY Program. The occurrence of *Acinetobacter* spp. increased moderately, occurrence of *E. coli* was decreased moderately. Prevalence rates of our most commonly isolated strains were closest to the ones isolated in ICU of UHO (in percentage), except for *Proteus* spp. The rate of *Acinetobacter* spp. was 100% higher at CMH,

while the rate of *E. coli* was, in opposite, 50% lower.

Important information acquired from MYSTIC and partially from SENTRY program is that the resistance of observed gram-negative bacteria has not virtually changed in any of the ICUs during the time of the study. Actually the resistance has decreased in some cases. Resistance of *Klebsiella* spp. to ceftazidime is an exception. The resistance of *Klebsiella* spp. and *P. aeruginosa* to ciprofloxacin has increased as well. Resistance of gram-negative pathogens with the highest rate of prevalence to selected antibacterial drugs at CMH during 1998–2002 is shown in Table 4. It is worth mentioning that the resistance situation at CMH during the investigated period was instable. In some cases it was substantially different in comparison with other departments.

When comparing the resistance of *E. coli*, *K. pneumoniae* and *P. aeruginosa* strains, acquired at CMH with the resistance status of Košice Hospital, Slovakia, in 1998 (Siegfried & Kme‰ovÁ, 1998), we found that the resistance of *E. coli* was the same in both hospitals at that time. However, the resistances of *Klebsiella* spp. and *Pseudomonas* spp. were almost 100% higher at CMH as those at Košice Hospital.

By comparing the representatives of *Enterobacteriaceae* family within our hospital during the last five years we found that the efficiency of penicillin and cephalosporin antibiotics worsens at *Citrobacter* spp., the efficiency of *Enterobacter* spp. remains roughly the same and in other cases, efficiency increases moderately. We observed the biggest increase in efficiency in *E. coli* strains (Tab. 4). The situation is similar in the group of aminoglycosides. Resistance to trimetroprim+sulfonamide and ciprofloxacin increased within the studied time in some cases by 100%.

Concerning the *Acinetobacter* spp., resistance to cephalosporins of the fourth generation has increased markedly (25%), resistance to meropenem increased by 15%, to trimetoprim+sulphonamide by 50%. In opposite, ciprofloxacin is almost 100% more efficient to *Acinetobacter* spp. than it used to be five years ago.

In 2002, *Pseudomonas* spp. showed lower resistance to piperacillin (21%) compared to 1998, to piperacillin+sulbactam (11%) and ceftazidime (12%), but higher resistance to ciprofloxacin (51%). Our results showed a little bit higher resistance to piperacillin-tazobactam, ceftazidime, meropenem, and amikacin in comparison with the

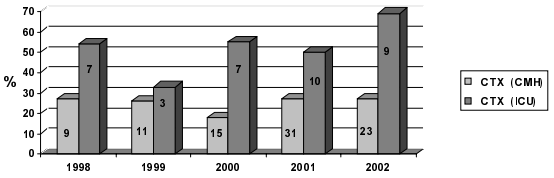

Fig. 1. Comparison of resistance of *Klebsiella* spp. to cefotaxime during the time of the study. In the columns, there are total numbers.

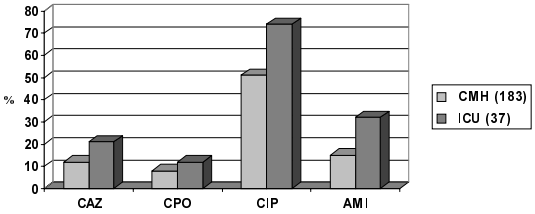

Fig. 2. Resistance to *Pseudomonas* spp. in % (2002).

results of national study on *P. aeruginosa* in Slovakia during 1998–2001.

Common characteristics of our findings and knowledge, acquired from MYSTIC, SENTRY and ICU of UHO, are that we also recorded an increase in the number of ciprofloxacin-resistant strains, e.g. resistance in *Pseudomonas* spp. is doubled compared to the above-mentioned studies. In our hospital this resistance was 51% (75% at ICU). We did not record any adverse results from ICU UHO regarding an increase of resistance to ceftazidime. In opposite, the resistance has decreased by 50% compared to 1998.

Observation of resistance shows that sometimes there are great differences between regions, hospitals and even departments (Wise & Andrews, 1998). This fact can be documented through stable values in resistance development. Our study showed that values obtained for the entire hospital are not the same as those for particular departments (Fig. 1). Also the resistance of *Pseudomonas* spp. to selected common antibiotics (Fig. 2) was, for example, at ICU ($n = 37$) higher by 50–100% in comparison to CMH ($n = 183$).

Nowadays *Pseudomonas* spp. is one of the problem strains at ICU. It is gram-negative pathogen number one (39%). Its prevalence during the years of the study was 31–46%. As expected, we recorded one of the highest increases of multiresistance in *Pseudomonas* spp. (Fig. 3).

Within the hospital, the highest resistance was found in 2002 in P*seudomonas* spp. (35%),

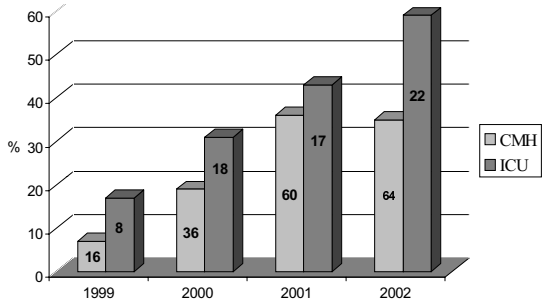

Fig. 3. The increase (in %) of the multi-resistant *Pseudomonas* spp. strains.

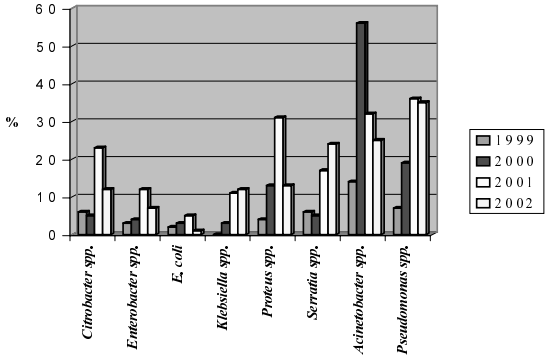

Fig. 4. Share of multi-resistance of particular microorganisms.

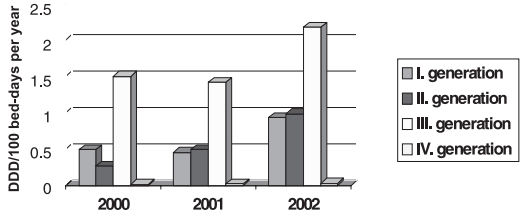

Fig. 5. Consumption of cephalosporins at CMH within the last three years.

A*cinetobacter* spp. (25%), *Serratia* spp. (24%), *Proteus* spp. (13%), and *Klebsiella* spp. and *Citrobacter* spp. (both 12%). Multi-resistance of *Enterobacter* spp. and *E. coli* was 7% and 1%, respectively. Figure 4 shows an increasing share of multi-resistance within the last years.

From the total 183 analyzed *Pseudomonas* spp. in 2002 in CMH, there were 64 multi-resistant strains, out of which 22 strains (34%) were from the ICU; 45% were isolated from respiration tract, 18% from urine and 37% from other materials delivered for analysis to microbiology department. Concerning the 83 *Klebsiella* spp., there were found 11 multi-resistant strains, out of which 7 were acquired at ICU representing 64%. 4 *Klebsiella* spp. strains were isolated from respiration tract, 2 from urine and 7 from other materials. Similarly, out of 121 *Enterobacter* spp. strains, there were 8 multi-resistant ones, one half being acquired at ICU. From the above results it is obvious that the multi-resistance is mainly an environmental problem with the highest selection pressure of antibiotics.

As a consequence of multi-resistance, the consumption of more expensive wide-spectrum antibiotics and reserve antibiotics increases. This fact can be seen in Figure 5 on the consumption of cephalosporin.

Anti-microbial resistance in hospitals represents not only medical but also an economical problem. It makes several cheaper antibiotics unusable. It often leads to longer hospitalization and the patient has to be prescribed more efficient and more expensive antibiotics. Increasing consumption of cephalosporins within the last three years also supports this argument. For example, consumption of cephalosporins of I generation in CMH in 2002 compared to 2001 rose by 106%, that of II generation by 100%, III generation by 53% and IV generation by 50% (Fig. 5).

Such an increase in consumption creates environment with strong selection pressure of cephalosporins, which supports the spread of resistant and multi-resistant bacterial strains affecting patients secondarily (Kolar et al., 2001). Both, the increase of resistant strains of *Klebsiella* spp. (Fig. 4) as well as the increase of *Enterobacter* spp. and *E. coli*, can support and prove this statement.

In summary, we demonstrated that antibiotics resistance increase is problem of our hospital, too. The presented data show great differences between departments of our hospital. The increase in consumption of cephalosporins creates environment with strong selection pressure. The high incidence of reduced antibiotic susceptibility suggests that more effective strategies (barrier contact measures, restriction of cephalosporins, antibiotic cycling in ICUs) are necessary to control the selection and spread of resistant organisms.

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