ORIGINAL ARTICLE

TRENDS IN ANTIMICROBIALS CONSUMPTION AND ANTIMICROBIAL RESISTANCE IN AN INFECTIOUS DISEASES HOSPITAL FROM THE SOUTH-EASTERN REGION OF ROMANIA

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Abstract

The aim of this study was to compare the antimicrobial preparations consumption with the resistance of the main bacterial species isolated in patients admitted to the Infectious Diseases Clinical Hospital "Sf. Cuv. Parascheva" of Galați. The bacterial strains have been identified in various pathological products (pharyngeal/nasal exudates, urine culture, coproculture, wound secretions, prosthetic devices/catheters, otic and conjunctival secretions, blood cultures, and puncture liquids) between 2013 and 2015 period. The antibiotics consumption in this hospital decreased for all antibiotics categories except for glycopeptides, whose usage doubled as a result of the increasing incidence of *Clostridium difficile* infections. The most often used class of antibiotics was that of penicillins, followed by cephalosporins and fluoroquinolones. The resistance of the isolated strains to antimicrobial agents increased, expressing resistance phenotypes of clinical and epidemiological significance, of which we can mention the increased incidence of the methicillin-resistant *Staphylococcus aureus* (MRSA) average 33.7%, and the production of extended-spectrum beta-lactamases (ESBL) in *E. coli* (12.7%) and in *Klebsiella spp.* (39.2%) in relation to the usage of cephalosporins of the third generation.

Rezumat

Scopul acestui studiu a fost acela de a compara consumul de produse antimicrobiene, în paralel cu rezistenţa principalelor specii bacteriene izolate la pacienții internați în Spitalul Clinic de Boli Infecţioase "Sf. Cuv. Parascheva" Galați, Romania. Tulpinile bacteriene au fost identificate din diferite produse patologice (exsudat farigian/nazal, uroculturi, coproculturi, secreţii ale plăgilor, dispozitive protetice-catetere, secreţii otice/conjunctivale, hemoculturi, lichide de puncţie) în perioada 2013 - 2015. Consumul antibioticelor în spital a fost descrescător pentru toate clasele de antibiotice cu excepția glicopeptidelor a căror utilizare s-a dublat ca urmare a creşterii incidenţei infecţiilor cu *Clostridium difficile.* Cea mai utilizată clasă de antibiotice a fost cea a penicilinelor, urmată de cefalosporine şi fluorochinolone. Rata rezistenţei la agenţii antimicrobieni pentru tulpinile izolate a fost crescută, acestea exprimând fenotipuri de rezistenţă de importanţă clinică şi epidemiologică, dintre care se remarcă incidenţa crescută a tulpinilor de stafilococ auriu rezistent la meticilină (MRSA) în medie (33,7%) şi producerea de beta-lactamaze cu spectru extins (ESBL) la *E. coli* (12,7%) şi la *Klebsiella spp.* (39,2%) în legătură cu utilizarea de cefalosporine de generaţia a treia.

Keywords: antibiotics, microbial resistance, infectious diseases, hospital infections

Introduction

The unregulated use at the large scale of antibiotics has determined the increase of incidence of antibiotics resistant bacteria, which is a major complication in the treatment strategy of bacterial infections [9]. The number of infections caused by multi-resistant microorganisms is rising, which leads to longer periods of hospitalization, and higher rates of morbidity and mortality, as well as higher costs. High resistance rates (RR) to antibiotics have been reported by WHO among pathogen bacteria [13], and the most recent European surveillance report issued by EARS-Net (European Antimicrobial Resistance Surveillance Report Network) provides information with regard to the general increase of antimicrobial resistance, in particular for Gram-negative bacteria [7].

Information on antibiotics consumption and resistance of the microbial strains may contribute to the drawing of resistance and antibiotics consumption maps with applicability in elaborating regional and national strategies of antibiotic prescription both in hospitals and ambulatory.

At the same time, the global surveillance of antibiotics resistance, as part of the European Antimicrobial Resistance Surveillance Network (EARS-Net) programme warns us in regard to the importance of implementing local studies or national surveillance programmes in view of highlighting the circulating resistance phenotypes, with the aim of guiding the empiric antibacterial therapy in clinical situations which require the initialisation of early antibacterial therapy.

Materials and Methods

A retrospective study for the 2013 - 2015 period has been conducted, concomitantly with the determination of the antimicrobials consumption and analysing the antibiotics sensitivity of the bacterial strains from patients admitted to the Infectious Diseases Clinic Hospital "Sf. Cuv. Parascheva" Galați, Romania.

The antibiotics consumption, which represents the frequency of using an antimicrobial, has been taken from hospital's pharmacy, in reference to penicillins (benzilpenicillin, ampicillin, amoxicillin, including amoxicillin-clavulanate acid), 2nd - 4th generations of cephalosporins (ceftriaxone, ceftazidime, cefoperazone, cefotaxime, cefuroxime), glycopeptide (vancomycin, teicoplanin), carbapenems (imipenem, meropenem, ertapenem), aminoglycosides (gentamicin), fluoro-quinolones (ciprofloxacin, moxifloxacin). The antibiotic consumption is expressed in DDD (defined daily dose) adjusted per 100 days- bed with the AMC (a programme to calculate antimicrobial consumption) Tool 1.5.0. Software. DDD is the antimicrobial agent standard dose for an adult as defined by the World Health Organisation (WHO) [14].

The bacterial strains have been identified in various pathological products (pharyngeal/nasal exudates, urine culture, coproculture, prosthetic devices, wound secretions, otic and conjunctival secretions, blood cultures, and puncture liquids). Gram staining has been applied to positive samples, as well as cultures for the identification and testing the antibiotic resistance. The biochemical identification of the microbial strains has been performed by using the multi-test systems (TSI (triple sugar iron), MIU (mobility, indol, ureasis), MILF (mobility, indol, lizindecarboxilasis, fenilalanindezaminasis) and Simmonscitrate) [1] and semiautomatic systems (API (analytical profile index) micro-test).

Testing the antibiotic sensitivity has been performed in the hospital laboratory by using the diffusimetric method through interpretative reading and phenotype identification of some mechanisms of resistance to beta-lactam antibiotics (synergism and antagonism tests for highlighting the extended-spectrum beta-lactamases) [2]. Depending on the zone diameter of bacterial growth inhibition (antibiotic impregnated disks, incubated 24 h at 37°C) and on the basis of standard tables of critical points, the isolates have been classified into sensitive, intermediate or resistant, according to Clinical and Laboratory Standards Institute (CLSI) (2013, 2014, 2015). The reference strains used for the quality control of the antibiogram were: *S. aureus* ATCC 25923, *E. coli ATCC* 25922, *Ps. aeruginosa ATCC* 27853, *Streptococcus pyogenes* ATCC 19615 [3, 4, 5].

The proportion of the resistant strains has been calculated by relating the number of isolated resistant strains to the total number of strains of the same species. The statistical analysis of data was performed with SPSS applications (Statistical Package for Social Science v.16.0).

In order to compare the two variables (antibiotics consumption and bacterial resistance), we used Pearson correlation, where the significance threshold was of $0 < p < 1$ with a confidence interval of 0.7 ± 1.

Results and Discussion

Antibiotics consumption. The total antibiotics consumption during the three years surveyed was of 162.333 defined daily dose (DDD)/bed-days, with a year by year decrease, from 63.8 to 42.8 (Table I). The most used antibiotics class was penicillin, with 32% of the total consumption in hospital, followed by cephalosporins 29%, fluoroquinolones 19% and aminoglycosides 14%; the least used were glycopeptides and carbapenemes with 3% each (Figure 1). The European Surveillance of Antimicrobial Consumption Network (ESAC-Net) collects data of the antimicrobials community consumption from 29 EU states and antimicrobial hospital consumption from 19 EU states. According to ESAC-Net, the antimicrobial consumption varies from one country to the other, and the antimicrobials most frequently used in hospital were 3rd generation cephalosporins and carbapenems, while penicillins were predominantly used in the community [6].

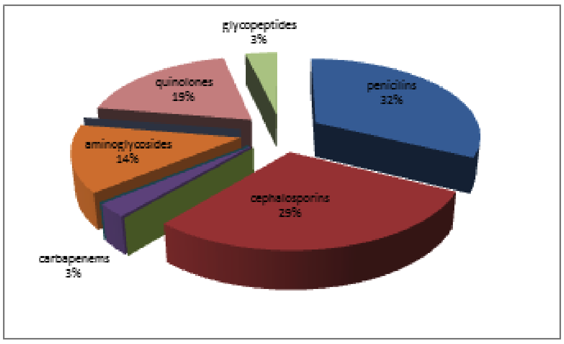

Figure 1.
Proportion of different classes of antimicrobials used in hospital

Table I

Trends in antimicrobials consumption expressed as DDD/100 bed-days during 2013 - 2015

	Penicillins	Cephalosporins	Carbapenems	Aminoglycosides	Quinolones	Glycopeptides	Total
2013	17.797	19.037	1.802	9.606	14.593	1.037	63.872
2014	20.912	15.031	1.621	6.474	9.088	2.531	55.657
2015	13.828	12.804	1.011	6.121	6.677	2.369	42.810
	52.537	46.872	4.434	22.201	30.358	5.937	**162.333**

During the 2012 - 2014 interval, Romania had one of the greatest antibiotics consumptions in the EU, 30.9 - 30.4 DDD/1000 inhabitants/day, outrun only by Greece, and for the years 2013 - 2014, the antibiotics consumption was of 32.47 and 31.16 DDD/inhabitants/day [6]. At national level, the most frequently used antimicrobial class was also that of penicillin [10], almost two times more than hospital consumption (55% *vs.* 32%), while the second most frequently used class, 2nd - 4th generation cephalosporins was used two times less than hospital consumption (15% *vs.* 29%). Quinolones were more frequently used at hospital level than at national level (19% *vs.* 11%). The increased consumption of glycopeptides, especially of vancomycin, was noted in 2014 as a result of the emergence and expansion of *Clostridium difficile* associated disease (CDAD) [12], consecutive to the antibiotic consumption in other hospitals. The decrease of antimicrobial consumption may be determined by the increase in the proportion of patients suffering from chronic viral diseases, whose treatment does not require antibiotic administering, considering that the patients' number was not significantly different from one year to the next, and also by the better use of antibiotics, in compliance with the national and international guidelines.

The present study represents a starting point in monitoring the antibiotics consumption and reporting the microbial resistance at the hospital level, in compliance with ECDC recommendations. For this undertaking not to be a singular and useful effort, it is necessary that regional surveillance includes not only sentinel-hospitals but all health units using antimicrobials.

Resistance. During the three surveillance years, out of 3120 non-repetitive isolated cases (Figure 2), negative Gram bacilli (2156) were most frequently isolated (*E. coli* 44%, *Klebsiella spp*. 8,14%, *Pseudomonas spp*. 7,4%), followed by *Staphylococcus spp*. 24%.

The bacterial strains isolated in our hospital presented patterns of natural (constitutive) resistance characteristic to their specific species, but also acquired antibiotic resistance, according to the isolation source of the respective strains.

Testing the sensitivity to ß-lactam of the *Staphylococcus aureus* strains revealed that 93.5% of these strains were penicillin resistant; for oxacillin, resistance decreased from 43% in 2014 to 34% in 2015. The prevalence of oxacillin resistance was below that reported by Romania, of 54%, the highest in the EU [7]. Penicillin resistance is most often caused by the modification of penicillin-binding protein (PBP) and extremely rarely by the beta-lactam production, which translates clinically in lacking benefits from the associations of penicillin and beta-lactam inhibitors compared to aminopenicillins [10, 11]. The significant oxacillin resistance rate reflects the diversity of the circulating *Staphylococcus aureus* strains, but also differences in using the antibiotics in a hospital environment, and the ineffectuality of the politics of monitoring the nosocomial infections [8].

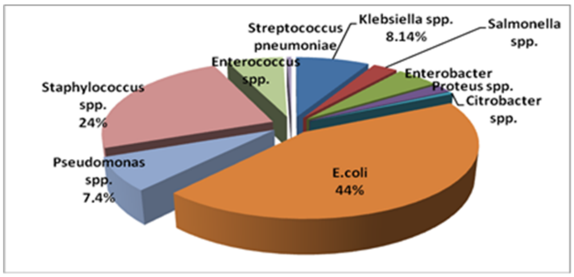

Figure 2.
Distribution of infectious agents isolated in hospital

Enterococcus spp. presents a decrease of penicillin resistance from 27% in 2013 to 15% in 2015 (Table II). All strains of *Streptococcus pneumoniae* presented in our study 100% sensitivity to penicillin, while being also sensitive to ceftriaxone, compared to 15.6% resistance reported for Romania [7]. The decreasing percentages of positive Gram cocci are in concordance with the decrease of penicillin consumption in hospital from 20.912 in 2014 to 13.878 DDD/100bed-days in 2015 (Table I).

For aminoglycosides, a decrease in the percentage of strains *Klebsiella spp*. and *Proteus spp.* was noted (36% *vs.* 28% and respectively 42% *vs.* 27%) (Table II), which is significantly different from the situation at the national level, which reports 67.3% resistance for *Klebsiella pneumoniae* [7]. Pearson correlation between antibiotic consumption and bacterial resistance to aminoglycosides in *Klebsiella spp*. and *Proteus spp.* is significant (Table III). The results obtained are in agreement with the theoretical data regarding the microbial resistance to antibiotics, especially in the case of *Klebsiella,* which develops multiple resistance to antimicrobials by plasmid genes acquisition. Resistance rate to fluoroquinolones of the *Klebsiella pneumoniae* strains is in continuous increase in all European countries. For Romania, the reported percentage is of 66.5%, [7] significantly higher than the resistance acquired in hospital (46.3%).

E. coli, the most frequent *Enterobacteriaceae* isolated in hospital, recorded an increasing resistance both to aminoglycosides and to fluoroquinolones.

Table II

Proportion of resistance markers in the analysed strains

Resistant strains %	**Antibiotics**								
	Penicilin G	Oxacilin	Amoxicilin/ Ac. clavulanic	Cefuroxime	Cefotaxime	Imipenem	Meropenem	Gentamicin	Ciprofloxacin
E. coli	*	*	50.8	16.0	12.7	7.3	2.4	12.4	35.6
Klebsiella spp.	*	*	64.4	41.4	39.2	9.9	22.8	32.6	46.3
Proteus spp.	*	*	63.0	51.6	39.3	18.3	30.6	36.7	37.4
Enterobacter spp.	*	*	100.0	100.0	39.7	14.3	*	12.0	25.7
Citrobacter spp.	*	*	78.6	60.6	47.0	0.0	*	35.2	36.0
Salmonella spp.	*	*	*	*	*	*	*	*	1.1
Staphylococcus aureus	93.5	33.7	*	*	*	*	*	31.0	30.6
Pseudomonas spp.	*	*	*	*	*	6.5	*	44.6	49.6
Enterococcus spp.	2.3	*	*	*	*	*	*	*	*
Streptococcus pneumoniae	0.0	*	*	*	*	*	*	*	*
Streptococcus spp.	0.0	*	*	*	*	*	*	*	*

The resistance rate to 3rd generation cephalosporins (cefotaxime) obtained is inscribed within 39% for *Klebsiella spp./Proteus spp.* and 12% for *E. coli* (Table II). RR to 3rd generation cephalosporin is in constant increase, reaching a rate of 73.7% reported by Romania [7] for *Klebsiella pneumonia* and of 29.4% for *E. coli*, which proves the wide dissemination of ESBL, both at community level and in hospital. At the same time, a positive correlation was noted for *E. coli* in the case of cefotaxime (Table III).

The increased usage of carbapenems in treating infections with *Enterobacteriaceae* secreting ESBL or gram-negative non-fermentative bacteria has determined the emergence of resistance to them, especially in the cases of *Klebsiella spp.* and *Pseudomonas spp.* (to imipenem 9.9% *vs.* 6.5%) (Table II). These values are below the maximum reported by Romania to carbapenems, 31.5% *vs.* 58.5%. In the case of Pearson correlation with *Klebsiella spp.*, the value of significance is positive for imipenem (Table III).

Table III

Pearson correlation between antibiotic consumption and microbial resistance to antibiotic therapy

Resistant strains %	**Antibiotics**							
	Penicillin G	Oxacillin	Amoxicillin/ Ac. clavulanic	Cefotaxime	Imipenem	Meropenem	Gentamicin	Ciprofloxacin
E. coli	*	*	**0.842**	**0.868**	-0.756	-0.122	-0.986	-0.946
Klebsiella spp.	*	*	**0.948**	0.281	**0.980**	0.122	**0.792**	-0.270
Proteus spp.	*	*	0.520	0.280	**0.748**	0.122	**0.910**	**0.948**
Enterobacter spp.	*	*	0.000	0.279	**0.985**	*	-0.913	**0.859**
Citrobacter spp.	*	*	-0.924	0.234	0.000	*	**0.976**	0.566
Salmonella spp.	*	*	*	*	*	*	*	**0.873**
Staphylococcus aureus	-0.955	-0.869	*	*	*	*	**0.770**	**0.760**
Pseudomonas spp.	*	*	*	*	-0.759	*	0.254	**0.721**
Enterococcus spp.	-0.617	*	*	*	*	*	*	*
Streptococcus pneumoniae	0.000	*	*	*	*	*	*	*
Streptococcus spp.	0.000	*	*	*	*	*	*	*

This microorganism represents a major public health problem in what the bacterial resistance to antibiotics is concerned, as it may produce further carbapenemases, subsequently transmitting to other *Enterobacteriaceae* the genetic material which codifies them [10].

This study has its limitations due to its retrospective nature, and because it was carried out in one infectious diseases hospital only, which may be less representative for general hospitals. A study conducted at the level of more hospitals would improve the representativeness.

Conclusions

The antibiotics consumption in the assessed hospital decreased for all antibiotics classes except for glycopeptides, whose usage doubled as a result of the increased incidence of *Clostridium difficile* infections.

The most frequently used antibiotics class was that of penicillins, followed by cephalosporins and fluoroquinolones.

The resistance rate to antimicrobial agents for the isolated strains was below the rate reported at the national level, significant correlations having been obtained for Gram-negative bacteria and the consumption of aminoglycosides and 3rd generation cephalosporins.

The surveillance of antimicrobial consumption concomitantly with the assessment of antibiotic resistance at the local and regional level may contribute in a more precise appreciation of both phenomena and in the institution of a better antimicrobial therapy practice, not only in hospitals, but also within the community.

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