The prevalence and resistance patterns of *Pseudomonas aeruginosa* in a tertiary care hospital in Kosovo

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SUMMARY

Pseudomonas aeruginosa is a Gram-negative bacterium that continues to a leading cause of opportunistic nosocomial infections. The rapid increase in drug resistance in clinical isolates of this pathogen is a worldwide concern. The aim of this study was to investigate the distribution rate, prevalence and resistance patterns of *P. aeruginosa* in clinical specimens from the University Clinical Centre of Kosovo (UCCK). During a three-year period, 553 *P. aeruginosa* isolates were collected from patients admitted to a variety of UCCK units. The *P. aeruginosa* isolates were identified using standard laboratory procedures, and the susceptibility of the isolates to antimicrobial agents was investigated using the Kirby-Bauer disk diffusion assay according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) (2013-2015) guidelines. *P. aeruginosa* was the second most frequently isolated pathogen. The isolation rate of *P. aeruginosa* was 7.6%, 10.1% and 8.6% in 2013, 2014 and 2015, respectively. Most clinical samples were from ICU (380, 68.7%). There was a statistically significant difference between ICU and non-ICU ($p<0.05$). *P. aeruginosa* isolates were most frequently isolated from the respiratory tract (323, 58.4%). The rate of resistance against most of the tested antimicrobials has increased, especially for carbapenems. Imipenem resistance was 25.2%, 26.5%, and 37.7% and meropenem resistance was 20.1%, 23.4%, and 36% in 2013, 2014 and 2015, respectively. This study provides important data on current antimicrobial resistance, and the results demonstrate that the resistance rates are progressively increasing. There is an urgent need to emphasise the prudent use of antibiotics and strictly adhere to the concept of "reserve drugs" to minimise the misuse of available antimicrobials. The acquisition and analysis of prevalence and resistance data will be an important tool to identify targets for quality improvement in Kosovo and will support the preparation of guidelines and protocols for the prudent use of antibiotics.

Keywords: *Pseudomonas aeruginosa*, resistance patterns, ICU.

INTRODUCTION

Pseudomonas aeruginosa is an opportunistic pathogen that can cause life-threatening infections in patients with immunological system defects, including burn patients, especially in developing countries [1]. *P. aeruginosa* is one of the leading causes of hospital-acquired infections, in particular for patients admitted to the ICU. Most infections caused by *P. aeruginosa* are often severe, life-threatening and are untreatable because of the high resistance to antimicrobial agents and the lack of new drug development [2].

Antibiotic resistance is a worldwide problem of major importance [3]. *P. aeruginosa* is intrinsically resistant to several antibiotics because of the low permeability of its outer membrane, the constitutive expression of various efflux pumps, and the production of antibiotic-inactivating enzymes (*e.g.*, cephalosporinases) [4]. The increasing resistance of *P. aeruginosa* to numerous antibiotics,

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as a result of excessive antibiotic administration, is now leading to the accumulation of antibiotic resistance, antibiotic cross-resistance, and the appearance of multidrug-resistant forms of *P. aeruginosa* [5].

Carbapenems remain the main antimicrobial class for the treatment of infections due to multidrug-resistant *P. aeruginosa*, but the development of carbapenem resistance may significantly compromise their efficacy [6]. Infections caused by either *P. aeruginosa* or *A. baumannii* strains that produce a carbapenemase pose an enormous challenge because these strains are very frequently multi-resistant and are often only susceptible to colistin [7]. The bactericidal antibiotic colistinhas not been commonly used because of its neurotoxic and nephrotoxic effects, but recently it has been found to be effective against MDR *P. aeruginosa* strains [8].

Antimicrobial resistance is one of the major challenges in the health care in Kosovo, and it is important to note that there is no functional system for supervision of antibiotic consumption and resistance at the national level. For many years in Kosovo, antibiotics have been used without restrictions not only in ICUs but also in other hospital wards and ambulatory care facilities. For a decade in Kosovo, all antimicrobials have been available in pharmacies without a physician's prescription [9]. Knowledge regarding *P. aeruginosa* in Kosovo hospitals is lacking.

Ongoing surveillance of *P. aeruginosa* resistance against antimicrobial agents is fundamental for monitoring trends in susceptibility patterns and to appropriately guide the clinician in choosing empirical or directed therapy [10]. The aim of our investigation was to assess the distribution rate, prevalence and antimicrobial resistance patterns of *P. aeruginosa* in UCCK, as the only tertiary care center in Kosovo with 2100 beds, for a period of three years.

PATIENTS AND METHODS

Study design and sample collection

This retrospective cross-sectional study was conducted in the Department of Microbiology of the National Institute of Public Health of Kosovo. During the three-year period of January 2013 - December 2015, a total of 553 *P. aeruginosa* isolates were analyzed in the Laboratory of Nosocomial Infection and Antimicrobial Resistance, which is the only laboratory for microbiological analyses for UCCK. All *P. aeruginosa* strains from reliable clinical specimens from hospitalized patients in different units in the UCCK were evaluated. The patient clinical data, including age, sex, ICU location (Adult Intensive Care and Neonatology Intensive Care) or non-ICU (Surgery, Pulmonology, Infectious Diseases, Paediatric unit, etc.), and their antimicrobial resistance profile were obtained. Ethical approval to undertake the study was obtained from the Ethical Committee of Faculty of Medicine in Prishtina. Identification of *P. aeruginosa* was accomplished using standard laboratory techniques, observing the colony characteristics on blood agar and MacConkey agar plates, biochemical tests (oxidase, urease, motility and sugar fermentation tests), and the Vitek automatic system 2 (bioMérieux-France).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by disk diffusion tests on Mueller-Hinton (MHA) agar using antibiotic discs. The bacterial colonies were picked up with a sterile loop, suspended in peptone water, and the turbidity of the suspension was adjusted to a 0.5 McFarland standard. The suspension was then spread on the surface of the MHA plate using a sterile cotton swab [11].

The results were interpreted as susceptible, intermediate or resistant according to the criteria recommended by European Committee on Antimicrobial Susceptibility Testing Clinical Breakpoint (EUCAST 2013-2015) [12]. The following antimicrobials were tested: ceftazidime 10 µg, cefepime 30 µg, ciprofloxacin 5 µg, gentamicin 10 µg, amikacin 30 µg, tobramycin 10 µg, piperacillin 10 µg, piperacillin-tazobactam 30 µg, imipenem 10 µg and meropenem 10 µg. Cartridges of antimicrobial-containing disks were obtained from Mast Diagnostic (UK).

Statistical analysis

Statistical analyses were performed using Statistical Package for Social Sciences (SPSS). The data were analyzed in terms of the mean, percentage, standard deviation and the Chi-square test. A statistically significant difference was considered for a $p<0.05$ at a 95% confidence interval.

RESULTS

During the three years of surveillance, a total of 6146 samples yielded significant growth. Gram-negative bacilli were identified in 2819 (45.8%) of the samples, and *P. aeruginosa* was the second most frequently isolated pathogen in 553 (19.6%) samples. Table 1 illustrates the patient characteristics and sample distribution of *P. aeruginosa*. The highest number of samples was from the ICU (380, 68.7%), and the other 173 (31.2%) samples were from non-ICU (other units). There was a statistically significant difference between the ICU and non-ICU ($p<0.05$). Themale: female ratio was 65.2:34.7. The isolates were most frequently recovered from tracheostomy tubes (225, 40.6%), followed by endotracheal aspirate (121, 21.9%), wounds (97, 17.5%), blood culture (37, 6.6%), punctate of pleural liquid (29, 5.2%), thoracic and abdominal drain swab (19, 3.4%), cerebrospinal liquid (17, 3%) and catheter tip (14, 2.5%). As noted in Table 2, the rate of resistance increased significantly against tested antimicrobials from 2013 to 2015: cefepime 31.6% to 64.5%;

Table 1- Patients' characteristics and sample distribution of *P. aeruginosa* in 2013-2015.

Patients' characteristics	*2013*		*2014*		*2015*	
Mean age (in years)	39.15		38.90		35.10	
	N	*%*	*N*	*%*	*N*	*%*
Male	88	76.5	168	68.2	105	54.6
Female	27	23.4	78	31.7	87	45.3
Clinical settings						
ICU patients	80	69.5	184	74.8	116	60.4
Non-ICU patients	35	30.4	62	25.2	76	39.5
Total	115	100	246	100	192	100
Site						
Tracheostomy tubes	46	40.0	112	45.5	68	35.4
Endotracheal aspirate	19	16.5	53	21.5	49	25.5
Wound	20	17.4	47	19.1	30	15.6
Blood	15	13.0	12	4.9	10	5.2
Thoracic and abdominal drain swab	8	7	4	1.6	7	3.6
Punctate of pleural liquid	4	3.5	8	3.3	17	8.9
CSF	2	1.7	5	2.0	3	1.6
Catheter tip	1	0.9	5	2.0	8	4.1
Total	115	100	246	100	192	100

ICU- Intensive Care Unit. CSF - Cerebrospinal fluid.

Table 2 - Resistance patterns of *P. aeruginosa* in 2013-2015.

Year	*Isolates No.*	*CAZ %*	*FEP %*	*GN %*	*AK %*	*TOB %*	*CIP %*	*PIP %*	*PIP/ TZP %*	*IMI %*	*MRP %*
2013	115	59.8	31.6	47.2	38.3	35.9	32.8	56.2	26.6	25.2	20.1
2014	246	37.3	44.9	59.4	59.8	60.5	55.5	65.4	18.8	26.5	23.4
2015	192	42.0	64.5	56.6	52.7	54.5	45.0	68.4	44.1	37.7	38.3

CAZ: ceftazidime, FEP: cefepime, GN: gentamicin, AK: amikacin, TOB: tobramycin, CIP: ciprofloxacin, PIP: piperacillin, PIP-TZP: piperacillin-tazobactam, IMI: imipenem, MRP: meropenem.

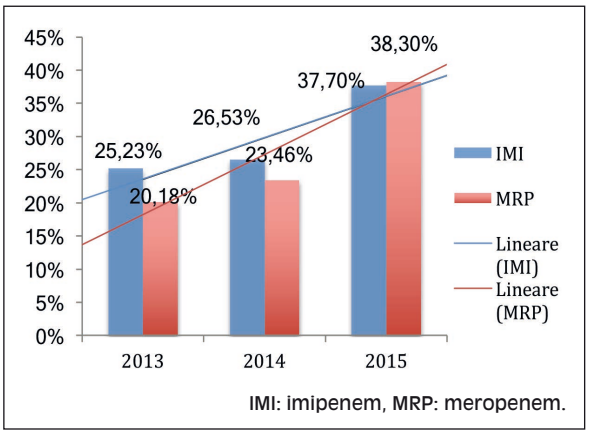

Figure 1 - Carbapenem resistance of *P. aeruginosa* in 2013-2015.

gentamicin 47.2% to 56.6%; amikacin 38.3% to 52.7%; tobramycin 35.9% to 54.5%; ciprofloxacin 32.8% to 45%; piperacillin 56.2% to 68.4%; and piperacillin-tazobactam 26.6% to 44.1%. A slight decrease in *P. aeruginosa* resistance to ceftazidime was found (59.8% to 42.0%). The most effective antibiotics were the carbapenems imipenem and meropenem with resistance rates at 29.8% and 18%, respectively, among the 553 *P. aeruginosa* isolates. Figure 1 shows the carbapenem resistance rate during 2013-2015.

In our study, susceptibility testing towards colistin was not routinely performed for each sample, but was conducted only in some strains that were resistant to all of the other tested antibiotics. On this basis, only 11 (1.98%) *P. aeruginosa* isolates that were tested against colistin were sensitive. Testing was performed with Etest (bioMérieux) since there was no EUCAST breakpoint for colistin using the diffusion method during the study period.

DISCUSSION

Because of the widespread use of antibiotics, especially in developing countries, the resistance profiles of many microorganisms are changing [13]. Laboratory-based surveillance is essential for the detection and control of drug-resistant pathogens. Despite this fact, we believe that documenting antimicrobial resistance, especially carbapenem resistance, is crucial as these strains often cause outbreaks in ICU settings [14]. ICUs have been clearly identified as endemic settings for *P. aeruginosa* [15]. Intensive care patients are at a particular risk of infection because of the debilitating effects of prolonged hospitalization and the application of medical equipment (airways, catheters, etc.) [16]. A multicenter study placed *P. aeruginosa* as the most common pathogen recovered in the ICU [17]. Our study is consistent with these studies, as the majority of isolates were also from the ICU.

Epidemiological studies on the prevalence and antimicrobial resistance in different geographical settings provides useful information and contribute to the global picture of antimicrobial resistance. According to EARSS data for 2014, high percentages of resistance in *P. aeruginosa* were reported, especially from eastern and south-eastern parts of Europe. The national percentages of resistant isolates ranged from 4.4% (the Netherlands) to 58.5% (Romania). Significant increases in resistance trends were observed for three countries (Germany, Hungary, and Slovakia) [18]. As reported in many studies, *P. aeruginosa* isolates are resistant to an increasing variety of antibiotics, including fourth generation cephalosporins. High levels of beta-lactam resistance have been reported in the United States, Europe, and South America [19]. Ceftazidime and cefepime are the most frequently prescribed third and fourth generation cephalosporins, respectively. Among beta-lactams, resistance to cefepime is significant in our study (31.6% in 2013, 44.2% in 2014, and 64.5% in 2015), and resistance to ceftazidime has decreased slightly (59.8% in 2013, 37.3% in 2014, and 42% in 2015). The differences in the antimicrobial resistance rate usually relate to the frequency of use and prescribing practices of the hospital.

Bayani et al. found that the resistance rate of *P. aeruginosa* to amikacin, ceftazidime, cefepime, imipenem, and ciprofloxacin was 53.3%, 43.3%, 40%, 40%, and 33.3%, respectively, and the prevalence of *P. aeruginosa* resistant isolates has increased [20]. Another study reported the following rates of resistance to cefepime 64.8%, piperacillin 45%, ciprofloxacin 38.9%, levofloxacin 36.1%, gentamicin 37.3% and amikacin 30% [21]. The present study showed that *P. aeruginosa* was highly resistant to aminoglycosides, including amikacin (52.7%), gentamicin (56.6%), and tobramycin (54.5%). This result contrasts with one of our previous studies,

which claims that amikacin has the highest sensitivity against *P. aeruginosa* [22]. However, based on the results of the present study, we cannot say the same as these antibiotics demonstrated considerable resistance that has increased during the last three years to almost double their initial values. In the present study, piperacillin-tazobactam showed high resistance of 26.6% to 44.1%.

For *P. aeruginosa* infections, carbapenem may be the only antibiotic choice. Thus, the emergence of carbapenem resistance is a serious concern [23]. In various studies across the world, varying rates of resistance from 4-60% have been reported for imipenem and meropenem [24]. Another survey found that resistance to imipenem was 19%, while other studies have reported low rates (5.8% and 9%) and high rates (38.6%) of resistance to imipenem [25].

The current study shows an increase of *P. aeruginosa* carbapenem resistance from 2013 to 2015 for imipenem (25.2% in 2013, 26.5% in 2014, and 37.7% in 2015) and meropenem (20.1% in 2013, 23.4% in 2014, and 38.3% in 2015). Similar increasing rates of imipenem resistance in *P. aeruginosa* (10.2% in 2013, 31.6% in 2014, and 22.1% in 2015) were reported in Croatia [26]. Unfortunately, the past two decades have seen a marked decline in the discovery and development of novel antibiotics and a remarkable increase in resistance to those that are currently available. In particular, there is substantial concern worldwide with the mounting prevalence of infections caused by multidrug-resistant Gram-negative bacteria, especially *P. aeruginosa*, *A. baumannii*, and *Klebsiella pneumoniae*. For these species, polymyxins are sometimes the only available active antibiotics. When the use of beta-lactams, aminoglycosides or quinolones is ineffective, the polymyxins, especially colistin, serve as the final alternative treatment [27].

A similar situation exists in Kosovo where beta-lactams, aminoglycosides, fluoroquinolones, carbapenems and colistin remain the available antibiotics for the treatment of *P. aeruginosa* infections. In the future, it is necessary to perform further studies related to the activity and efficacy of colistin, especially for multi-resistant strains of *P. aeruginosa*.

In the targeted Gram-negative area, two new cephalosporin-beta-lactamase inhibitor combinations, ceftazidime-avibactam and ceftolozane-tazobactam, are approved that are differentiated in their activity only on a molecular level of specific resistance mechanisms. They may add valuable options in specific cases of multidrug or even extensively drug resistant *Pseudomonas* infections based on extensive microbiological diagnostic efforts and results [28]. Unfortunately, these new drugs are not introduced in Kosovo market.

To the best of our knowledge, this is the first clinical investigation of *P. aeruginosa* from Kosovo and was conducted to identify the antibiotics to which *P. aeruginosa* has developed resistance during the last three years. The resistance to antipseudomonal antibiotics was found to be high, and these data suggest that resistance is progressively increasing in our country. In this study, the antimicrobial susceptibility of *P. aeruginosa* was tested using the Kirby-Bauer disc diffusion method. However, further investigation is necessary, and molecular studies are essential to identify the resistance mechanisms.

CONCLUSION

P. aeruginosa was found ata high frequency in patients treated in ICUs. The emergence of antibiotic resistance in *P. aeruginosa* has been increasing in Kosovo. The dissemination of carbapenem resistance is worrisome and calls for the implementation of surveillance studies as well as the judicious use of antibiotics. State and national level antimicrobial policy and guidelines should be introduced to preserve the effectiveness of antibiotics in Kosovo.

Conflict of interest. The authors have no conflicts of interest to disclose.

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