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Retrospektywna ocena patogenów i ich lekowrażliwości wykrywanych w posiewach moczu chorych hospitalizowanych w Klinice Nefrologii w roku 2010

Streszczenie

Wstęp. Zakażenia układu moczowego (ZUM) są najczęściej występującą kliniczną postacią zakażeń szpitalnych u dorosłych pacjentów, stanowiąc około 40-50% zakażeń chorych hospitalizowanych. Znajomość mikroflory uczestniczącej w zakażeniach układu moczowego u pacjentów szpitalnych oraz jej wrażliwości na leki stosowane w praktyce nefrologicznej jest niezbędna dla wdrożenia skutecznej terapii.

Cel. Celem pracy była identyfikacja uropatogenów wyizolowanych w roku 2010 z próbek moczu od pacjentów hospitalizowanych w Klinice Nefrologii SPSK4 oraz określenie wrażliwości in vitro uropatogennych szczepów na leki przeciwbakteryjne.

Materiał i metoda. Analizowane próbki moczu w liczbie 539 pochodziły od pacjentów leczonych w Klinice Nefrologii SPSK4 w Lublinie. Próbki moczu poddawano analizie przy użyciu aparatu Vitek 2 Compact firmy BioMeriux.

Wyniki. Liczba badań dodatnich wynosiła 41.9%. Wśród Gram ujemnych patogenów dominowały szczepy *Escherichia coli* – 40.7 %, następnie *Enterobacter cloacae* – 6.5%, *Klebsiella pneumoniae ssp pneumoniae* – 6.5%, *Proteus mirabilis* – 6.5%, *Pseudomonas aeruginosa* – 5.2%, inne szczepu Gram ujemne – łącznie 11.3%. Bakterie Gram dodatnie z grupy *Enterococcus faecalis* wyhodowano w 7.3% próbek moczu. Inne stwierdzone bakterie Gram dodatnie stanowiły łącznie 12.4% wyhodowanych szczepów.

Wnioski. Przeprowadzona analiza drobnoustrojów wskazuje na coraz większy udział bakterii Gram ujemnych nietypowych, o wielolekowej oporności na antybiotyki, w patogenezie powikłanych infekcji dróg moczowych. Duży problem terapeutyczny stanowią zakażenia florą Gram dodatnią, o ograniczonych możliwościach terapeutycznych, ze względu na szybki rozwój szczepów wielolekoopornych. W przypadku podejrzenia infekcji układu moczowego u chorego hospitalizowanego trudno przewidzieć uropatogen i jego antybiotykowrażliwość, dlatego niezbędne jest wykonanie posiewu moczu i wdrożenie celowanego leczenia. Ze względu na konieczność stosowania coraz nowszych, droższych generacji antybiotyków w leczeniu ZUM, niezbędna jest ponowna wycena procedury leczenia szpitalnego infekcji dróg moczowych, która jest niedoszacowana i nie pokrywa rosnących kosztów terapii.

Słowa kluczowe: infekcja dróg moczowych, antybiotykooporność, wewnątrzszpitalne ZUM.

Retrospective analysis of pathogens isolated in urinary cultures taken from patients hospitalized at the Department of Nephrology in 2010 and their antimicrobial resistance

Abstract

Introduction. Urinary tract infections (UTIs) are ones of the most common clinical type of infectious diseases among patients treated in hospitals, constituting about 40-50% of all infections among inpatient adults. Knowledge about microorganisms causing UTIs among hospitalized patients and their antibiotic sensitivity is essential to order effective antibacterial therapy.

Aim. The purpose of the study was to indentify microorganisms, that were isolated in urinary samples taken from patients hospitalized in Nephrology Department in 2010 and to assess their antimicrobial resistance in vitro.

Material and methods. The 539 examined urinary samples were taken from patients hospitalized in Nephrology Department in Lublin in 2010. The urinary samples were analyzed with Vitek 2 Compact device of BioMeriux.

Results. From 539 urinary samples taken from patients hospitalized in Nephrology Department in 2010, 226 (41.9%) were positive. Among Gram negative bacteria *Escherichia coli* dominated – 40.7%, subsequently strains of *Enterobacter cloacae* – 6.5%, *Klebsiella pneumoniae ssp pneumoniae* – 6.5%, Proteus mirabilis – 6.5%, *Pseudomonas aeruginosa* – 5.2% and other Gram negative bacteria made up totally 11.3% of isolated strains. Gram positive bacteria *Enterococcus faecalis* were cultured in 7.3% of urinary samples and other Gram positive bacteria constituted totally 12.4 %.

Conclusions. The examination of isolated microorganisms indicates rising amount of untypical, multiantimicrobial resistant Gram negative bacteria in pathogenesis of complicated UTIs. Gram positive bacteria cause big therapeutic problem with restricted therapeutic options bring out by rapid development of multidrug resistance. In case of UTI suspicion in inpatient person, it is difficult to predict pathogen and its antimicrobial resistance, therefore urinary culture is essential to order effective treatment. As a result of necessity of using more and more expensive , new antibiotics in treatment of UTIs, it seems to be indispensable to value again the procedure of treatment of UTIs of hospitalized patients, because it is underestimated and doesn't cover rising costs of therapy.

Key words: urinary tract infection, antimicrobial resistance, inpatient UTIs.

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INTRODUCTION

Urinary Tract Infections (UTIs) are the most common infectious diseases and one of the most frequent reason of medical appointment and hospitalisation. UTIs are also the most common clinical type of infectious diseases among patients treated in hospitals, constituting about 40-50% of all infections among inpatient adults and more than 90% among patient hospitalized in nephrology and urology wards [1].

Urinary tract infection consists in the presence of microbes (mainly bacteria, less frequently fungi or viruses) in the urinary tract above the bladder sphincter. UTI is diagnosed mainly on the basis of characteristic clinical symptoms, but the final confirmation is the isolated microorganism from the urine in titre higher than conventionally accepted limit, i.e. the diagnosis of so called significant bacteriuria. A significant bacteriuria in 90% of typical cases, when urine is collected using the method of mid-stream, shall be:

- $>10^3$ CFU/ml, with concomitant cystitis symptoms (pain, burning feeling during urination, frequent urination, reddish urine)
- $>10^4$ CFU/ml, if there are concomitant symptoms typical for acute pyelonephritis (fever above 38° C, abdominal pain that radiates along the flank towards the back, vomiting)
- $>10^5$ CFU/ml without clinical symptoms and leukocyturia and in two subsequent cultures – symptomless bacteriuria [2].

The most frequent cause of UTI is bacteria *Escherichia coli* (in majority of non complicated infections and in about half of complicated ones). Other bacteria are isolated from urine of patients more often in complicated than in non-complicated cases: *Klebsiella sp., Proteus mirabils, Enterococcus sp., Pseudomonasaeruginosa* [3].

Depending on the presence of subjective and objective symptoms there are distinguished:

- asymptomatic bacteriuria
- clinically overt urinary tract infection, which can only relate to lower urinary tract or spread to the upper part of the urinary tract.

Both the infection of the lower and upper urinary tract can be:

- uncomplicated
- complicated.

Uncomplicated urinary tract infection occurs almost exclusively in women with normal urogenital system, and without disturbance of local and systemic defence mechanisms and is caused by microorganisms typical of urinary tract infections.

Uncomplicated infection usually does not require hospitalization and can be effectively treated in outpatient department.

Complicated urinary tract infections are:

- infections of urinary tract in men
- infections of urinary tract in women with anatomical or functional disorders of urine outflow or with impairment of systemic or local defensive mechanisms.
- urinary tract infections caused by atypical pathogens.

In clinical practice, we deal almost exclusively with complicated infections that are caused by most gram-negative rods (GNR). Infection usually occurs in ascending mode. The reason is the endogenous microflora (own or acquired as a result of colonization during hospitalization) or exogenous microflora from the hospital environment or derived from other patients. Approximately 80% of nosocomial UTIs is associated with the use of urinary catheters. Treatment of these infections is extremely difficult [4].

In the treatment of UTIs in the nephrological wards there are many problems associated with the increasing contribution in infections of Gram-negative non-uropathogenic bacteria, i.e. non-fermenting bacteria, the increasing resistance to used antibiotics, the ability of bacteria to maintainin a hospital environment, tendency to chronic infections and the possibility of serious complications, such as urosepsis or renal failure, resulting in prolongation of hospitalization and increased costs of treatment. In addition, antibiotic therapy in nephrological patients is complex and it must consider renal function, which is often incorrect as well as many concomitant illnesses. Often, due to increasing resistance to antibiotics, the infections must be treated with potentially nephrotoxic antibiotics at doses adjusted to renal function [5].

Knowledge of bacterial and fungal microflora involved in urinary tract infections in hospitalized patients and its antimicrobial resistance used in nephrology practice is necessary in order to implement effective therapy.

AIM

The purpose of the study was to indentify uropathegens isolated in urinary isolates taken from patients hospitalized in Nephrology Department of Independent Public University Hospital No.4 in 2010 and to assess their in vitro antimicrobial resistance.

MATERIAL AND METHODS

The urinary samples (isolates) in the number 539 were taken for analysis from patients hospitalized in Nephrology Department in Lublin. The urinary ‘midstream’ samples were collected to sterile container.

In case of suspected UTI from catheterised patients, urinary catheter was removed, and urine was collected with the new catheter for up to30 minutes since its introduction. In patients with the need of using catheter chronically, urine was collected directly from the catheter, after disinfecting the injection site. Within an hour the urine was delivered to the Laboratory of Microbiology. The urinary isolates were analysed with Vitek 2 Compact device of BioMeriux, that is fully automatic non-invasive system to identify microorganisms and assess their antimicrobial susceptibility. The device consists of analyser, computer and printer. The analyser includes filler station, load station for inserting/removing cassettes, barcode reader, incubation-measurement station, waste collection bin.

The filler station consists of filling chamber, in which all AST test cards are inoculated with bacteria suspension. While the test cards are present in the load station, the yare subjected to incubation at35.50C. The biochemical reactions that occur in the hollows of cards are automatically analysed.

After incubation are port for each card is generated. The incubation time is 2-12hours.The report includes the identification of the strain and the antimicrobial resistance given as the MIC value and category (S-susceptible, R resistant, I-intermediate).

The apparatus is equipped with two types of cards: identification and antimicrobial susceptibility testing cards. Identification cards include microwells with substrates designed for biochemical identification. Identification cards allow for performing standard and specialized biochemical tests, automatically read by an optical scanner.

Antimicrobial susceptibility testing cards contain wells with the respective concentrations of the antibacterial preparation mixed with culture medium. Bacterial suspension causes hydration of the antibiotics substrate. The computer determines whether the inoculum in the well is positive (bacterial growth) or negative (no growth) based on the level of light intensity by optical scanner. Growth progress is assessed in each well every hour. This way obtained data are used to determine the MIC of antimicrobial drug and identify resistance mechanisms.

RESULTS

For the tests in 2010, urine samples (isolates) were obtained from 539 patients treated at the Department of Nephrology. The number of positive tests was 226 (41.9%). Among Gram negative pathogens *Escherichia coli* strains predominated – 40.7%, including a strain of *Escherichia coli ESBL*, then strains of *Enterobacter cloacae* – 6.5%, *Klebsiellapneumoniaessppneumoniae* – 6.5%, *Proteus mirabilis* – 6.5%, *Pseudomonas aeruginosa* – 5.2%, *Morganellamorganiisspmorganii* – 3.6%, *Serratiamarcescens* – 3.2%. Other Gram negative rods isolated from the samples accounted for a total of 4.5% of isolates (*Acinetobacterbaumannie,Citroba cterfreundi, Proteus vulgaris, Citrobacterkoseri, Klebsiellaoxytoca, Kliebsiellapneumoniae, Stenotrophomonasmaltophilia*).

Gram-positive bacteria (GPC) of *Enterococcus faecalis* were grown in 7.3% of urine samples. Other GPC identified were *Enterococcus faecium* strains – 4.4%, *Streptococcus agalactiae* – 3.6%, *Staphylococcus aureus* – 1.2%, and *Staphylococcus, Corynebacterium, Lactococcusgarvieae* – totally 3.2%.

In 3.6% of analyzed urine isolates fungi were grown – mainly *Candida albicans* – 2.8%, the rest were *Candida famata* and *Candida parapsilosis*.The results of in vitro susceptibility testing of uropathogenic strains to antimicrobial agents are summarized in the table. They describe the maximum of five drugs most active in vitro against uropathogens belonging to particular types of bacteria (Table 1-4).

DISCUSSION

Hospital infections and the related antibiotic therapy are one of the major problems of modern medicine. The hospital is a particularly favourable environment for living and spread of germs. The use of antibiotics leads to the development of various resistance mechanisms, then the selection of strains resistant in the environment. In the present study pathogens that cause urinary tract infections in patients treated at the Department of Nephrology in Lublin in 2010 and their susceptibility to antibacterial agents, were identified.

Gram negative rods (GNR) were isolated in total in 76.7% of the analysed isolates. The most frequently isolated uropathogene was *Escherichia coli* grown in 40.7% of the analysed material. The analysis of antimicrobial resistance showed the greatest in vitro sensitivityto carbapenems, aminoglycosides and third generation cephalosporins, especially in combination with sulbactam. Isolates had 63% resistance to the ciprofloxacin of fluoroquinolones groupapplied on an outpatient basis, often as a medicine of first line treatment of UTI, while as much as 82% resistance – to cefazolin–the first generation cephalosporin. The results indicate that susceptibility of *E. coli* to fluoroquinolones decreases, which is also confirmed by the observations of other authors [6-8]. Great care must be taken when prescribing this group of drugs as the first line drugs.

Other isolated GNRs: *Enterobacter cloacae*, *Klebsiellapneumoniaessppneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Morganellamorganiisspmorganii*, *Serratiamarcescens* – showed the greatest susceptibility to carbapenems, amino-glycosides, third generation cephalosporins, and resistance or very little sensitivity to fluoroquinolones, second-generation cephalosporinsand semi-synthetic penicillins.

Among the GNRs there are mechanisms of resistance to antibiotics, which can cause failure in infection treatment and selection as well as spread of multi-drug-resistance strains in a hospital environment. The rapid development of antibiotic resistance limits the possibility of "targeted" therapy with antibiotics. Bacteria of this group have the ability to form biofilms, which is a thin layer of polysaccharide on the surface of epithelium of the urinary tract or biomaterials (catheters), which hinders the access of antibiotic to receptor sites; sometimes it is the cause of recurrence and chronic persistence of UTI [9].

GNR found in the urine culture of patients from the Department of Nephrology showed the highest sensitivity to carbapenems and aminoglisides. The global studies on susceptibilityof *E.coli* to antibiotics also show a growing susceptibility to carbapenems [10]. The use of carbapenems (imipen, meronemaztreonam) significantly increases the cost of patient hospitalization and additionally some GNR, particularly *Pseudomonas aeruginosa*, can develop resistance to its activity during treatment. To prevent the development of resistance it is recommended to use carbapenems in combination with an aminoglycoside or a fluoroquinolone, which also causes a synergistic effect [11].

Aminoglycoside antibiotics are the most well-known nephrotoxic drugs, therefore the use of these drugs at nephrology wards must be extremely prudent, the doses used and the interval between them should take into account the endogenous creatinine clearance. The duration of treatment should be limited, preferably up to 7 days [12].

The second most common uropathogen isolated in patients treated at the Department of Nephrology in 2010, were Gram-positive cocci (GPC) *Enterococcus faecalis*, sensitive to semisynthetic penicillin and glycopeptide antibiotics. The second most commonly grown cocci – *Enterococcus*

TABLE 1. Total microorganisms identified.

Microocganisms identified		
Name of microorganism	Culture/ number of cultures	Identification in %
Escherichia coli	100/539	40.3%
Enterococcus faecalis	18/539	7.3%
Enterobacter cloacae	16/539	6.5%
Klebsiellapneuminiae	16/539	6.5%
Proteus mirablis	16/539	6.5%
Pseudomonas aeruginosa	13/539	5.2%
Enterococcus faecium	11/539	4.4%
Morganellamorganii	9/539	3.6%
Serratiamarcescens	8/539	3.2%
Candida albicans	7/539	2.8%
Streptococcus agalactiae	6/539	2.4%
Staphylococcus aureus	3/539	1.2%
Streptococcus agalactiae (Strep. Group B)	3/539	1.2%
Acinetobacterbaummanii	2/539	0.8%
Citrobacterfreundii	2/539	0.8%
Coagulase negative Staphylococcus	2/539	0.8%
Proteus vulgaris spp	2/539	0.8%
Staphylococcus epidermidis	2/539	0.8%
Candida famata	1/539	0.4%
Candida parapsilosis	1/539	0.4%
Citrobacterkoseri	1/539	0.4%
Corynebacteriumspp	1/539	0.4%
Enterococcus spp	1/539	0.4%
Escherichia coli ESBL	1/539	0.4%
Klebsiellaoxytoca	1/539	0.4%
Klebsiella pneumonia	1/539	0.4%
Lactococcusgarviae	1/539	0.4%
Staphylococcus haemolyticus	1/539	0.4%
Staphylococcus xylosus	1/539	0.4%
Stenotrophomonasmaltophilia	1/539	0.4%

TABLE 2. Antibiotic susceptibility of Gram-negative rods.

Name of microorganism	Antibiotic	Susceptibility		
		S	I	R
E.coli	cefoperazon/sulbactam	100%	0%	0%
	impipenem	100%	0%	0%
	amikacyna	97%	0%	3%
	cefazolina	82%	2%	16%
	ceftriakson	89%	0%	11%
Enterobacter cloacae	imipenem	100%	0%	0%
	meronem	100%	0%	0%
	amikacyna	77%	0%	23%
	ceftazydym	33%	33%	33%
	cefepim	23%	0%	77%

TABLE 2. ctnd

Klebsiellapneuminiae	doripenem	100%	0%	0%
	imipenem	100%	0%	0%
	meropenem	100%	0%	0%
	amikacyna	87%	7%	7%
	lewofloksacyna	50%	0%	50%
Proteus mirablis	meropenem	100%	0%	0%
	Piperacylina/ Tazobactam	88%	6%	6%
	amikacyna	71%	0%	29%
	Ampicylina/ Sulbaktam	50%	50%	0%
	ciprofloksacyna	44%	0%	56%
Pseudomonas aeruginosa	meropenem	100%	0%	0%
	Tikarcylina/ kwasklawulanowy	71%	0%	29%
	ceftazydym	67%	22%	11%
	aztreonam	64%	27%	9%
	amikacyna	62%	8%	31%
Morganellamorganii	amikacyna	100%	0%	0%
	ceftazydym	100%	0%	0%
	imipipenem	100%	0%	0%
	meropenem	100%	0%	0%
	ciprofloksacyna	44%	0%	56%
Serratiamarcescens	ceftazydym	100%	0%	0%
	doripenem	100%	0%	0%
	Imipenem	75%	0%	25%
	meropenem	71%	29%	0%
	amikacyna	50%	0%	50%

TABLE 3. Antibiotic susceptibility of Gram-positive bacteria.

Name of microorganism	Antibiotic	Susceptibility		
		S	I	R
Enterococcus faecalis	wankomycyna	100%	0%	0%
	tigecylina	100%	0%	0%
	linezolid	100%	0%	0%
	ampicylina	100%	0%	0%
	ciprofloksacyna	35%	0%	65%
Enterococcus faecium	tigecylina	100%	0%	0%
	linezolid	100%	0%	0%
	wankomycyna	64%	0%	34%
	nitrofurantoina	18%	36%	45%
	Chinupristina/ dalfopristina	82%	0%	18%
Streptococcus agalactiae	ampicylina	100%	0%	0%
	ciprofloksacyna	100%	0%	0%
	nitrofurantoina	100%	0%	0%
	tigecylina	100%	0%	0%
	wankomycyna	100%	0%	0%
Staphylococcus aureus	gentamycyna	100%	0%	0%
	rifampicyna	100%	0%	0%
	wankomycyna	100%	0%	0%
	tigecylina	100%	0%	0%
	fosfomycyna	100%	0%	0%

TABLE 4. Antibiotic susceptibility of fungi.

Name of microorganism	Antibiotic	Susceptibility		
		S	I	R
Candida albicans	amfoterycyna	100%	0%	0%
	flucytozyna	100%	0%	0%
	flukonazol	100%	0%	0%
	ketokonazol	100%	0%	0%
	vorikonazol	100%	0%	0%
Candida famata	amfoterycyna	100%	0%	0%
	flucytozyna	100%	0%	0%
	flukonazol	0%	100%	0%
	vorikonazol	100%	0%	0%
Candida parapsilosis	amfoterycyna	100%	0%	0%
	flucytozyna	100%	0%	0%
	flukonazol	100%	0%	0%
	vorikonazol	100%	0%	0%

faecium remained resistant to most applied antibiotics – showed sensitivity to glycopeptide antibiotics and the new-generation oxazolidine and glicylocyclines antibiotics group.

The cultured strains of Streptococcus were susceptible to semisynthetic penicillin and fluoroquinolones, while the Staphylococcus strains were significantly more resistant by showing sensitivity to amino glycosides, rifampicin, glycopeptides antibiotics. The results of this study indicate a growing contribution of GPC, with high antibiotic resistance in the pathogenesis of urinary tract infections, as confirmed by other authors [13].

In total GPC were isolated in 19.8% of urine samples. The growing contribution of GPC in the pathogenesis of urinary tract infection is also confirmed in reports of other authors [14].

Fungi, mainly Candida albicans, were detected in 3.6% of the isolates and were susceptible to most antifungal medicines.

CONCLUSIONS

1. The analysis of microorganisms isolated in urine cultures from patients hospitalized in 2010 indicates a growing contribution of atypical GNRs, with multidrug resistance to antibiotics in the pathogenesis of complicated urinary tract infections
2. A big therapeutic problem are infections with Gram positive flora, with limited therapeutic options due to a rapid growth of multi-drug-resistance strains.
3. In case of suspected urinary tract infection in a hospitalised patient, it is hard to predict the uropathogen and its resistance to antibiotics. Therefore it is necessary to perform urine culture and implement a targeted treatment.
4. Due to the need of using newer and newer, more expensive generation of antibiotics to treat UTIs, It is necessary to re-price hospital treatment procedures of urinary tract infections, which is understated and does not cover the rising costs of treatment.

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