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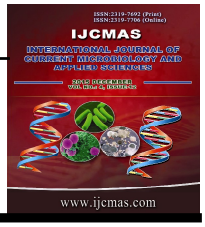

Original Research Article

Prevalence and Antibiogram of Multidrug resistant Uropathogenic Isolates of *Proteus mirabilis* in a Teaching Tertiary Care Hospital

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Keywords

Proteus mirabilis, Multidrug resistant, Uropathogens, Antibiotics, Urinary tract infections, Carbapenems

A B S T R A C T

Proteus mirabilis is a commonly isolated pathogen from patients suffering from complicated urinary tract infections. The study was conducted over a period of one and half year (January 2013 to June 2014). Clean catch midstream urine specimens or catheterized urine samples were collected in sterile containers and were processed by standard Microbiological techniques. Antimicrobial susceptibility testing was done according to CLSI guidelines. MIC was calculated by BD Phoenix automated system. The overall prevalence of *P. mirabilis* was 2.8% among culture positive urine samples. Antibiotic susceptibility profile of *P. mirabilis* showed that piperacillin/tazobactam, meropenem, cefotaxime, amikacin, aztreonam and ciprofloxacin were the most active antibiotics as 87% to 98% of the strains were susceptible while ampicillin, cefuroxime, ofloxacin and chloramphenicol were least effective as none to only 13% of the strains were susceptible. According to the pattern of antibiotic susceptibility in a health care institution, clinical microbiology laboratories should decide that among group A and B, which agents should be reported routinely and which agents should be reported selectively in consultation with infection control committee of the health care institution. Selective reporting minimise the selection of multidrug resistant strains by overuse of broad spectrum agents.

Introduction

Urinary tract infections (UTIs) are the most common bacterial infections among outpatients as well as inpatients and cause significant morbidity and health care costs. *Proteus mirabilis* is a well known uropathogen causing complicated UTIs in patients having indwelling urinary catheters and structural abnormalities of the urinary tract. This organism has a predilection for the kidney, causing cystitis, pyelonephritis and production of urinary stones which is a hallmark of infection with this organism. The ability of the *P. mirabilis* to cause stone formation is due to the presence of urease enzyme that hydrolyse urea into ammonia, causing local pH to rise with subsequent precipitation of magnesium ammonium phosphate (struvite) and calcium phosphate (apatite) crystals (Burall et al (2004).

P. mirabilis is generally susceptible to ampicillin, amoxicillin, piperacillin, broad spectrum cephalosporins, aminoglycosides and imipenem but it has intrinsic resistance to nitrofurantoin, tetracycline and colistin (Hara et al (2000). However, now a days due to increasing antibiotic resistance in *Enterobacteriaceae* the multidrug resistant (MDR) strains of *Proteus* species has also been reported worldwide. Production of Extended spectrum beta lactamases (ESBL) are one of the main mechanisms responsible for antibiotic resistance to beta lactams (penicillins, cephalosporins, aztreonam) and concomitant resistance to other groups of drugs causing emergence of MDR strains. Biofilm forming ability of the *P. mirabilis* further creates an environment leading to difficulty in antibiotic penetration and thus leading to antibiotic resistance. The different types of fimbriae expressed by the organism such as PmfA, PmfC, PmfD, PmfE and PmfF also play cardinal roles in colonization of urinary bladder and contribute to the pathogenesis of UTI among humans. Therefore, in view of increasing prevalence, routine detection of MDR strains of *Proteus mirabilis* is necessary in clinical microbiology laboratories for the timely and appropriate prescription of antibiotic therapy to improve patients's survival (Huang et al (2014), Cohen et al (2010).

The aim of present study was to obtain data on antibiotic resistance pattern of *P. mirabilis* cultured from urine specimens of patients attending urology outpatient department and patients admitted in urology ward with signs and symptoms suggestive of UTI and to know the prevalence of MDR isolates of *P. mirabilis.*

Materials and Methods

Study Design

The present prospective study was conducted at the Microbiology Department of our teaching tertiary care hospital over a period of one and half year (January 2013 to June 2014). Clean catch midstream urine specimens or catheterized urine samples were collected in sterile containers from patients of all age and sex groups attending the urology outdoor with complaints of UTIs and patients admitted in urology ward. These urine samples were received in Microbiology department where they were processed by standard conventional Microbiological techniques.

Identification and Antimicrobial Susceptibility Testing

Microscopic examination of urine samples was done to detect the presence of polymorphs (pyuria), red blood cells, and bacteria. Culture was put on blood agar and MacConkey agar semiquantitatively with the help of standardized loop and was incubated at 37^0C overnight. Presence of significant bacteriuria was indicated by colony count $\geq 10^5$ cfu/ml. Swarming on blood agar and non lactose fermenting, oxidase negative colonies on MacConkey agar were further processed for identification of *P. mirabilis* by standard biochemical reactions (Collee et al (1996). Antimicrobial susceptibility testing was done on Mueller Hinton agar (MHA) by Kirby-Bauer disc diffusion method according to Clinical Laboratory Standard Institute (CLSI) guidelines (CLSI (2006). The following antimicrobial discs (Hi-media, Mumbai, India) with their concentration given in parenthesis were used.

Group A: ampicillin (10µg), gentamicin (10µg).

Group B: amikacin (30µg), amoxicillin clavulanic acid (20/10µg), piperacillin tazobactam (100/10µg), cefuroxime (30µg),

cefepime (30µg), cefotaxime (30µg), ciprofloxacin (5µg), levofloxacin (5µg), imipenem (10 µg), meropenem (10µg), trimethoprim-sulfamethoxazole (1.25/23.75µg).

Group C: ceftazidime (30µg), aztreonam (30µg), chloramphenicol (30µg), tetracycline (30µg)

Group U: ofloxacin (5µg), norfloxacin (10µg), nitrofurantoin (300µg)

Group O: ceftizoxime (30µg), netilmicin (30µg), doxycycline (30µg)

All the confirmed isolates of *P. mirabilis* by biochemical reactions were simultaneously tested in automated BD Phoenix system present in our laboratory by which minimum inhibitory concentration (MIC) of these drugs were calculated.

Results and Discussion

A total of 70 non-duplicate, consecutive isolates of *P. mirabilis* were obtained during study period. The overall prevalence of *P. mirabilis* was 2.8% among culture positive urine samples. The sex wise distribution showed that males were more commonly affected 48/70 (68.5%) than females 22/70 (31.4%). The people of older age groups (51-80) were more susceptible to complicated UTI 50/70 (71.4%) as compared to younger age group people 20/70 (28.5%) (table 1). Antibiotic susceptibility profile of *P. mirabilis* showed that piperacillin/tazobactam, meropenem, cefotaxime, amikacin, aztreonam and ciprofloxacin were the most active antibiotics as 87% to 98% of the strains were susceptible while ampicillin, cefuroxime, ofloxacin and chloramphenicol were least effective as none to only 13% of the strains were susceptible (table 2). Antibiogram of *P. mirabilis* is shown in table 3.

UTI caused by *Proteus* account for 1-2% of community acquired infections. *Proteus* infections, if unrecognised can become established and cause major health problems. In our study, UTI caused by *P. mirabilis* is more common in elderly males. Complicated UTI was also found to be more frequent in older male patients in a study from past (Cernohorska (2011). The high incidence of UTI in elderly males can be due to prostate enlargement which causes bladder outlet obstruction predisposing males to urinary stasis and UTIs. In elderly people, complicated UTI is also more common due to genitourinary abnormalities, urolithiasis, dehydration and diabetes (McAllister et al (2014).

Worldwide data shows that there is an increasing resistance among uropathogenic strains of *P. mirabilis* to conventional drugs. Resistance has also emerged to newer, more potent antimicrobial agents. Study from Poland in 2013 reported that 40% of the uropathogenic *P. mirabilis* were resistant to ciprofloxacin and 64% were resistant to ceftazidime (Pirog et al (2013). Taiwan surveillance of antimicrobial resistance program showed that between 2002 to 2012, susceptibility of *P. mirabilis* to cefotaxime, ceftazidime and ciprofloxacin decreased significantly from 92.6% to 81.7%, 100% to 95.2% and 80.1% to 53.8% respectively (Wang et al (2014).

In our study, we tried to know the susceptibility results for most of the antibiotics as given in CLSI document for *P. mirabilis* but some of the antibiotic discs were not available during study period so, we were not able to test activity of tobramycin, ampicillin/sulbactam, ticarcillin/ clavulanic acid, cefotetan, cefamandole, cefoperazone, moxalactam, ertapenem, doxycycline and cefixime against *P. mirabilis*. These antibiotics were

also not present in panel of BD Phoenix automated system. This was one of the limitations of our study.

Group A drugs are those drugs which are considered appropriate for routine primary testing and routine reporting of results of these antibiotics are done for the specific organisms. Group B drugs are used for primary testing but they are reported selectively such as when the organism is resistant to antimicrobial agents of group A, in case of polymicrobial infection at one site, infections at multiple sites, patient having allergy to group A drugs. Group U includes those drugs which are used only for treating urinary tract infections (CLSI (2006).

In the present study, among group A drugs, all isolates showed uniform resistance to ampicillin (100%) and 19 isolates out of 70 were resistant to gentamicin (27%). Ampicillin is an orally administered drug but it was totally ineffective against *P. mirabilis* isolates. Gentamicin is an injectable drug, so, it is not convenient for the outdoor patients to administer it by own. This drug is also contraindicated in renal impairment which is more common in older age groups and in our study more than 70% of the patients were above 50 years of age. Therefore, testing and reporting of group B drugs was needed to guide urologists for prescribing appropriate therapy which could also be easy to administer by outdoor patients.

Owing to intrinsic resistance of *P. mirabilis* to nitrofurantoin and cotrimoxazole, both of these are the commonly used drugs for treatment of UTI, may become ineffective invivo inspite of the invitro susceptibility, therefore, resulting in treatment failure. Hence, oral treatment options for complicated UTI caused by *P. mirabilis* are limited, particularly if the strains are extended spectrum beta lactamase (ESBL) producer due to which they also showed concurrent resistance to trimethoprim and fluoroquinolones.

As shown in figure 1 that resistance to group B drugs was also quite high in gentamicin resistant isolates, out of 19 gentamicin resistant isolates, 6 (31.5%) were also resistant to five other group B drugs (amoxyclav, cefuroxime, levofloxacin, imipenem and cotrimoxazole), 5 (26.3%) were also resistant to four other group B drugs. Average resistance to group A, group B and group U drugs was 64.25%, 31.4% and 67.1% respectively.

Group C drugs are alternative or supplemental antimicrobial agents that may require testing in those institutions that harbour endemic or epidemic strains resistant to several of the primary drugs. Group O includes other antimicrobial agents that have a clinical indication for the organism but are not preferred for routine testing and reporting (CLSI (2006). Out of group C drugs, chloramphenicol is not routinely reported for uropathogens and for tetracycline *P. mirabilis* shows intrinsic resistance.

Among fluoroquinolones, ciprofloxacin and norfloxacin were most effective antibiotics showing 87.1% and 64.2% activity against *Proteus mirabilis* invitro while ofloxacin and levofloxacin were least effective. Fluoroquinolones are the most common antibiotics prescribed by clinicians as empiric treatment of UTI. Unrestricted use of fluoroquinolones as empiric therapy by clinicians before invitro antibiotic testing results is a factor causing increased resistance to these antibiotics in bacteria. The review of literature revealed that many studies in past has also reported high drug resistance in *P. mirabilis.*

Table.1 Age and Sex Wise Distribution of *P. Mirabilis*

Age group	Males n (%)	Females n (%)	Total
0-10	0 (0.0)	0 (0.0)	0 (0.0)
11-20	2 (4.1%)	2 (9.0%)	4 (5.7%)
21-30	0 (0)	8 (36.3%)	8 (11.4%)
31-40	2 (4.1%)	0 (0.0)	2 (2.8%)
41-50	4 (8.3%)	2 (9.0%)	6 (8.5%)
51-60	16 (33.3%)	2 (9.0%)	18 (25.7%)
61-70	4 (8.3%)	2 (9.0%)	6 (8.5%)
71-80	20 (41.6%)	6 (27.2%)	26 (37.1%)
Total	48 (68.5%)	22 (31.4%)	70

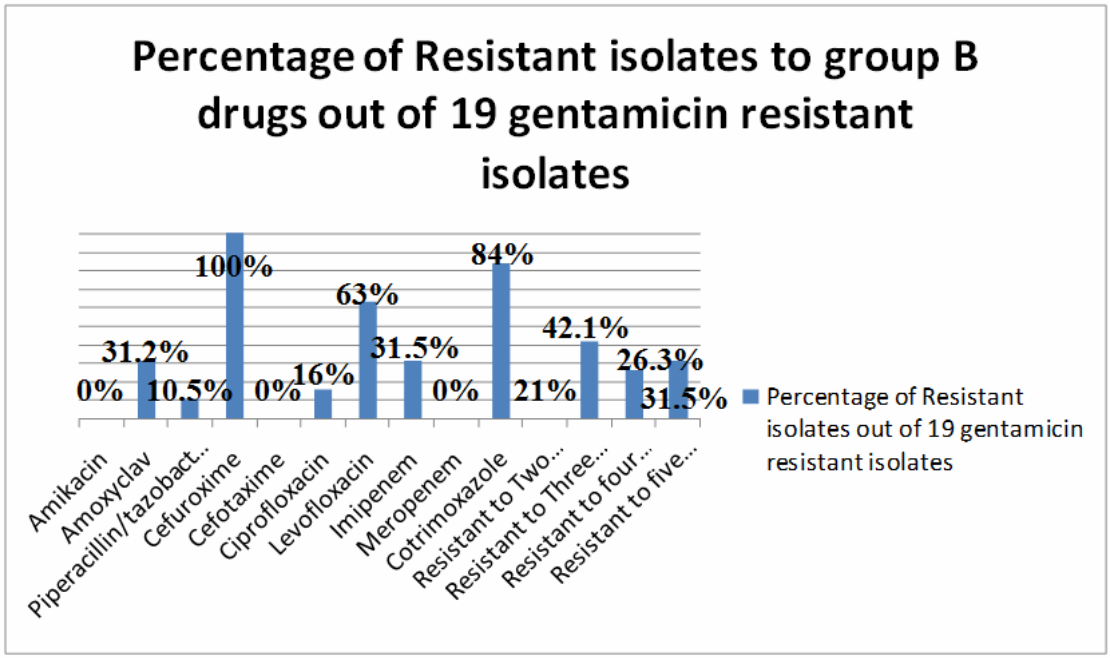
Table.2 Antibiotic Susceptibility Pattern of *P. Mirabilis* to Group A, Group B and Group U Drugs

Name of the drug		Number of susceptible isolates	Number of Resistant isolates
Group A	Ampicillin	0 (0.0%)	70 (100%)
	Gentamicin	51 (71.4%)	19 (28.5%)
Group B	Amikacin	62 (88.5%)	8 (11.4%)
	Amoxycillin/clavulanate	54 (77.1%)	16 (22.8%)
	Piperacillin/tazobactam	64 (91.4%)	6 (8.5%)
	Cefuroxime	09 (12.8%)	61 (87.1%)
	Cefotaxime	68 (97.1%)	2 (2.8%)
	Cefepime	40 (57.2%)	30 (42.8%)
	Ciprofloxacin	61 (87.1%)	9 (12.8%)
	Levofloxacin	35 (50%)	35 (50%)
	Imipenem	53 (75.7%)	17 (24.2%)
	Meropenem	67 (95.7%)	3 (4.3%)
	Cotrimoxazole	17 (24.2%)	53 (75.7%)
Group U	Ofloxacin	17 (24.2%)	53 (75.7%)
	Norfloxacin	45 (64.2%)	25 (35.7%)
	Nitrofurantoin	18 (25.7%)	52 (74.2%)
	Cefazolin	13 (18.6%)	57 (81.4%)
Group C	Ceftazidime	20 (28.5%)	50 (71.4%)
	Aztreonam	65 (92.8%)	5 (7.1%)
Group O	Ceftizoxime	38 (54.2%)	32 (45.7%)
	Netilmicin	60 (85.7%)	10 (14.2%)

Table.3 Antibiogram of *P. Mirabilis*

	Number of resistant isolates
Resistance to 2 different drugs	2 (2.9%)
Resistance to 3 different drugs	2 (2.9%)
Resistance to 5 different drugs	5 (7.1%)
Resistance to 6 different drugs	5 (7.1%)
Resistance to 7 different drugs	14 (20%)
Resistance to 8 different drugs	6 (8.5%)
Resistance to 9 different drugs	6 (8.5%)
Resistance to 10 different drugs	6 (8.5%)
Resistance to 11 different drugs	9 (12.8%)
Resistance to 12 different drugs	9 (12.8%)
Resistance to 14 different drugs	4 (5.7%)
Resistance to 16 different drugs	2 (2.9%)
Total number of isolates	70

Figure.1 Percentage of Resistance to Group B Drugs in Isolates which were Resistant to first line Drugs



Study from Meerut (India) in 2014 has reported that 35.7%, 57.1%, 14.3%, 28.57%, 21.4%, 78.5%, 14.3%, 28.5% of the *Proteus* strains were resistant to ciprofloxacin, ofloxacin, levofloxacin, amikacin, gentamicin, ceftazidime, cefotaxime, cotrimoxazole respectively (Prakash et al (2013). In our study, approximately 75-95% of the *Proteus* isolates were sensitive to carbapenems. Among aminoglycosides, amikacin was most effective drug as it was active against 88.5% of *Proteus* strains

invitro. Similar findings have been reported in past that imipenem was the most effective antibiotic with percentage of antimicrobial sensitivity of 91% against *Proteus* spp. Imipenem was followed by amikacin and aztreonam (61% and 47.7% sensitivity). Antimicrobial resistance for ampicillin and cotrimoxazole was more than 80% in the same study (Bahashwan et al (2013). Study from other region of world also reported that imipenem, ertapenem and amikacin showed 100% activity against *Proteus* while in the same study resistance to ampicillin, gentamicin, ciprofloxacin, cotrimoxazole was reported to be 71.4%, 57.2%, 40.4%, 88% respectively (Alhambra (2004).

Because of emergence of MDR strains of *P. mirabilis* in our hospital, selection of empiric antibiotics for UTI has become more challenging for clinicians. According to the pattern of antibiotic susceptibility in a health care institution, clinical microbiology laboratories should decide that among group A and B, which agents should be reported routinely and which agents should be reported selectively in consultation with the clinicians and infection control committee of the health care institution. Selective reporting improves the clinical relevance of test reports and minimise the selection of multidrug resistant strains by overuse of broad spectrum agents.

In conclusion, there is a paucity of data on antibiotic resistance pattern of uropathogenic *P. mirabilis* from North India. Our results show high degree of resistance to almost all antibiotics as compared to previously reported studies. All the isolates were resistant to at least three different antibiotics. Elderly patients with complicated UTI are often left with no oral antibiotic options. Administration of IV antibiotics requires hospitalization with close monitoring of renal functions, therefore, costs escalate rapidly when IV antibiotics are given. Therefore, the new strategies to manage UTI by *P. mirabilis* are needed to explore.

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