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Research Article

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Detection of antibiotic resistance pattern with ESBL producers and MRSA among the uropathogens at Tertiary Health Care Centre, North Bengal

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ABSTRACT

The objective of the present study was to determine distribution and antibiotic susceptibility pattern of bacterial strains isolated from patients suffering from UTI at tertiary health care centre in North Bengal, with special reference to ESBL and MRSA producers. This health care centre was chosen for the study as this centre is visited by patients from inside and outside the country. Moreover, this health care centre is also visited by patients from neighboring countries like western part of Bangladesh, Bhutan and Eastern Nepal. The present retrospective study was conducted from july 2013 to july 2014 where 457 uropathogens were isolated from 2090 consecutive urine samples. Automated identification and susceptibility (AST) system that analyzed MIC patterns was used. ESBL producers, their phenotypes and MRSA were identified. Results were analyzed using computer software, specifically designed to evaluate the results generated by the automated system. The most prevalent pathogens were Escherichia coli (48%) followed by Klebsiella spp (22%) and Pseudomonas aeruginosa (5%). Majority of the isolates (59%) were from females. Prevalence of ESBL and MRSA was found to be 33.26 % and 75% respectively. Higher than 80% resistance were observed for broad-spectrum penicillin with an increasing resistance to third generation cephalosporins and quinolone drugs. Tigegcycline was found to be effective against both gram negative and gram positive uropathogen. Daptomycin and Colistin was found to be drug of choice for both gram positive and for gram negative uropathogen respectively. The data highlights a serious need to monitor the current trend of growing antibiotic resistance. It indicates that it is imperative to rationalize the use of antimicrobials and employ them conservatively.

Keywords: *Urinary tract infection, uropathogens, Antibiotic Resistance, ESBL, MRSA etc.*

INTRODUCTION

Since the first description of plasmid-mediated extended spectrum beta lactamase (ESBL) in 1983, ESBL-producing gram-negative organisms have posed a significant threat to hospitalized patients due to their hydrolyzing activity against extended spectrum cephalosporins often employed in the treatment of hospital-acquired infections. Detection of organisms harboring ESBLs provides clinicians with helpful information. Treatment of infections caused by ESBL-producing organisms with extended-spectrum cephalosporins or aztreonam may result in treatment failure even when the causative organisms appear to be susceptible to these antimicrobial agents by routine susceptibility testing[24].

The β lactamase enzymes produced by the organisms break down the structural beta-lactam ring of β-lactam antibiotics. Many genera of gram negative bacteria possess a naturally occurring, chromosomally mediated β-lactamase and also some are plasmid mediated β-lactamases[24].

Urinary tract infections (UTI) are one of the most common infectious diseases seen in the clinical practice and community. In recent studies microbial species that cause urinary system infection are classified by their target sites, Such as urine infection (bacteriuria), bladder infection (cystitis), kidney infection (pyelonephritis), which can be asymptomatic or associated with symptoms[17,19]. It has been estimated that nearly 10% of the human population will experience a UTI during their life time[10]. UTI is the third most common cause of admission to hospitals in India. Gram negative bacteria are most often implicated in causing UTI[3]. Detection of ESBL producing organisms from urine samples will be valuable as this represents an epidemiologic marker of colonization.

It has been estimated that about 6 million patients per year are visited worldwide for UTI out of which around 30,000 are treated in the wards[5]. An estimate of patients suffering from UTI is around 150 million per annum across the Globe, which may rise to 75% in the female population by the age of 24, and 15–25% of this group will suffer from a relapse of this disease[15,16,20,25].

In eastern India, UTI is a common infection found among all ages from infants to elderly persons. However, studies on UTI and the susceptibility pattern of antibiotics in Eastern India are still underway, and there is extensive debate on the choice of antibiotics due to the lack of clear guidelines.

Knowledge of the etiology and antibiotic susceptibility pattern of the pathogen causing UTI is absolutely essential. The introduction of antimicrobial therapy has contributed significantly to the management of UTIs. However the main problem with current antibiotic therapies is the rapid emergence of antimicrobial resistance in hospitals and the community[13]. The resistance pattern of community acquired uropathogens has not been extensively studied in the Indian subcontinent[3,18]. No data concerning the antimicrobial resistance of bacteria isolated from UTIs from this part of the country that is North Bengal been documented till date.

It is important to realize that there may be marked differences between various geographic areas within a vast country like India. Since most UTIs are treated empirically the selection of antimicrobial agent should be determined not only by the most likely pathogen but also by its expected susceptibility pattern. Thus, knowledge of local antimicrobial susceptibility patterns of common uropathogens is essential for prudent empiric therapy of community acquired UTIs.

Nowadays, infectious pathogens are mostly resistant to several antibiotics, and this undermines the ability of antibiotics to control infections[14,21,29,30].

Hence, the present study was designed to study the current antibiotic susceptibility pattern among the uropathogens and to detect ESBL production and MRSA among them. The current study is of critical importance since it is useful for preparing the current antibiotic policy, in infection controlling policy and for detection and control of the outbreak of ESBL and MRSA producing organisms in a hospital. Additionally, the study also aimed at identifying possible resistance trends. As per electronic literature survey no such kind of study has been documented in the study area.

MATERIALS AND METHODS

Design & Setting

The data was taken from the WHONET software from the department of Microbiology, Tertiary health Care hospital, North Bengal during July 2013 to July 2014. Besides the people of North Bengal this health care centre is also visited by patients from nearby states like Eastern Bihar and whole Sikkim. Moreover, this health care centre is also visited by patients from neighboring countries like western part of Bangladesh, Bhutan and Eastern Nepal.

This was an analysis of data generated from the records of consecutive urine samples received in the laboratory from hospital's indoor and outdoor during the study period.

The anonymity of the patients was ensured. All data were retrospectively collected and de-identified when this was necessary to ensure patient confidentiality.

The study included all the patients who were admitted or visited the out-patient department in the hospital or health centre with symptoms of UTI during the study period and then had UTI confirmed further by positive urine culture reports. During this period a total of 2090 urine samples were collected. Majority of the samples were midstream clean catch urine followed by stream catheter, catheter site, catheter central, catheter peripheral, catheter permanent, catheter umbilical, urine bladder, urine clean voided , suprapubi caspirate, urine first voided, urine kidney, urine nephrostomy, urine non catherized and urine obtained from Foley's catheter.

Isolation of pathogen

The samples were observed carefully for adult parasite, consistency, blood, mucous, color and pH. In microscopic observation the samples were observed carefully for pus cells and red blood cells. Urine samples were cultured using a 1μmcalibrated loop onto Hichrome UTI agar plates. The samples were inoculated onto High Chrome UTI Agar (Hi Media, India) by semi quantitative method and incubated aerobically at 37ºC for complete 24 h incubation. The specimen yielding more than or equal to 10^5organisms/ml of urine was interpreted as significant**.** Isolates were identified on the basis of gram staining, colony morphology and standard biochemical tests. The isolates were further identified by vitek 2 instruments (VITEK 2 compact, Biomerieux).

Antibiotic Susceptibility Testing

All gram positive and the gram negative uropathogens were subjected for Antibiotic Susceptibility testing and the results were interpreted by modified Vitek 2 method automated system. The system included an Advanced Expert System (AES) that analyzed MIC patterns and detected the phenotype of organisms. Pure subcultures of QC and clinical organisms were suspended in aqueous 0.45% (wt/vol) NaCl to achieve a turbidity equivalent to that of a McFarland 2.0 standard (range, 1.80 to 2.20), as measured by the Densi Chek (bioMerieux) turbidity meter. Strain characterization and antimicrobial susceptibility testing were performed with the VITEK 2 automated system using the ID-GNB and AST-N280 and ID-GPC and AST- P628 cards for gram negative and gram positive bacteria respectively, in accordance with the manufacturer's instructions[11].

The VITEK 2 instrument automatically filled, sealed, and incubated the individual test cards with the prepared culture suspension. Cards were held at 35.5°C for 18 h, with optical readings taken automatically every 15 min. Based on these readings, an identification profile was established and interpreted according to a specific algorithm. The antimicrobial susceptibility testing card comprises various antibiotics which includes Ampicillin, Amoxicillin/ clavulanic acid, cefuroxime, cefuroxime/axetil, Ceftriaxone, Ciprofloxacin, Trimethoprim/Sulfamethoxazole, Pipercillin/ Tazobactum, Cefoperazone/Sulbactam, Cefepime, Imipenem, Meropenem, Amikacin, Gentamicin, Nalidixic acid, Nitrofurantoin, Colistin, Tigercycline for gram negative bacteria and for gram positive bacteria the testing card comprised the following antibiotics Benzyl penicillin, Oxacillin, Gentamicin, Ciprofloxacin, Levofloxacin, Erythromycin, Clindamycin , Linezolid, Daptomycin, Teicoplanin, Vancomycin, Tetracycline, Tigecycline, Nitrofurantoin, Rifampicin and Trimethoprim/Sulfamethoxazole. Final results were analysed using version 7.01 software, an AES specifically designed to evaluate the results generated by the VITEK 2 system**.**

ESBL and Methicillin Resistant Staphylococcus aureus (MRSA) Testing

Each isolate was tested using the VITEK 2 (software configuration version R07.01) system with the ESBL test panel with six wells containing three third generation cephalosporin, alone and in combination with clavulanic acid (CA). Growth in each well was quantitatively assessed by means of an optical scanner. The proportional reduction in growth in wells containing cephalosporin plus CA compared with those containing the cephalosporin alone was considered indicative of ESBL production. Quality control strains were included in each run. All phenotypic interpretations of ESBLs were reported as a positive ESBL screening result. Strains were reported as ESBL-negative whenever phenotypic interpretations other than ESBLs were proposed by the AES.

The Vitek (software configuration version R07.01)automated susceptibility testing system with a modified Gram-Positive Susceptibility (GPS) 106 Card (bioMerieuxVitek, Inc) were evaluated for their ability to detect oxacillin resistance in *Staphylococcus aureus*.

RESULTS

Microorganisms

Data from a total of 2090 consecutive urine samples were included in the study. Out of these, 1633 (78.2%) were sterile, 457(21.8%) showed significant growth.

Out of these positive cultures (n = 457), 391 gram negative rods and 42 gram positive cocci were isolated. Among gram negative rods major pathogens were *Escherichia coli* (56%) followed by *Klebsiella pneumoniae ss. Pneumoniae* (25%), *Pseudomonas aeruginosa* (6%), *Acinetobacter baumannii* (5%), *Citrobacter freundii* (1%), *Pseudomonas putida* (1%), *Proteus mirabilis* (1%), *Enterobacter cloacae* (1%), *Proteus rettgeri* (1%), *Klebsiella oxytoca* (1%), *Citrobacter koseri* (diversus) (1%). Among gram positive rods *Enterococcus faecalis* (32%) *Enterococcus sp* (20%), *Enterococcus faecium* (18%), *Staphylococcus aureus ss. aureus* (9%), *Staphylococcus haemolyticus* (9%), *Staphylococcus sciuri ss. lentus* (5%), *Staphylococcus epidermidis* (2%), *Staphylococcus saprophyticus ss. saprophytic* (2%) and *Staphylococcus warneri* (2%) were isolated.

Moreover, some of the fungal pathogen which was isolated was *Candida albicans, Candida tropicalis, Candida glabrata* and *Candida parapsilosis* (Table 1).

116 uropathogens were isolated from critical care unit (surgical and Medical care intensive units =73, Pediatric are intensive units=2, Neonatal care intensive units=0, High Dependency unit=41) and from non critical care (cabins=10, Surgical male and female ward=15 and 20 respectively, Medical male and Female ward= 39 and 52 respectively, emergency=3, nursery=2) (Table 2).

Table 1: All organisms isolated from urinary tract infected patient (457 isolates)

Mostly isolated uropathogens				
Organism	**No. of isolates**	**(%)**	**2013**	**2014**
Escherichia coli	220	48	75	145
Klebsiella pneumoniae ss. pneumoniae	100	22	48	52
Pseudomonas aeruginosa	24	5	9	15
Acinetobacter baumannii	20	4	9	11
Enterococcus faecalis	14	3	5	9
Occasionallv isolated uropathogens				
Candida albicans	9	2		9
Enterococcus sp.	9	2	9	
Enterococcus faecium	8	2		8
Candida tropicalis	6	1		6
Rarely isolated uropathogens				
Proteus mirabilis	4	1	2	2
Staphylococcus haemolyticus	4	1	2	2
Pseudomonas putida	4	1	3	1
Citrobacter freundii	4	1	3	1
Staphylococcus aureus ss. aureus	4	1	2	2
Enterobacter cloacae	4	1	2	2
Citrobacter koseri (diversus)	3	1		3
Proteus rettgeri	3	1	2	1
Klebsiella oxytoca	3	1		3
Staphylococcus sciuri ss. lentus	2	0	1	1
Candida glabrata	2	0		2
Stenotrophomonas maltophilia	1	0		1

Staphylococcus saprophyticus ss. saprophytic	1	0		1
Acinetobacter lwoffii	1	0	1	
Staphylococcus epidermidis	1	0		1
Sphingomonas paucimobilis	1	0	1	
Raoultella ornitholytica	1	0		1
Pseudomonas stutzeri	1	0	1	
Candida parapsilosis	1	0		1
Klebsiella sp.	1	0	1	
Staphylococcus warneri	1	0		1

Table 2: Distribution of uropathogens from different wards

	Locations	**No. of isolates**
1.	Critical care unit	116
2.	Semi critical	141

Distribution of uropathogen among gender

The distribution of uropathogen was checked in both males and females. The distribution was checked by checking the number of isolated organism from both male and female urinary tract infected patient. It was found that maximum number of uropathogens was isolated from females. A total of 184 isolates (enterobactereciae=117, gram negative =127, gram positive = 23) was isolated from males whereas a total of (enterobactereciae = 225, gram negative=229, gram positive=19) 267 isolates was isolated from females. Therefore, it was found that higher percentage of women (59%) to be suffering from UTI as compared to men (41%).

Antibiotic Susceptibility testing

Antibiotic susceptibility testing was performed for all the isolates. In general, tigegcycline was found to be effective against both gram negative and gram positive uropathogen. Daptomycin and Colistin was found to be drug of choice for both gram positive and for gram negative uropathogen respectively (Table 3 and 4).

Table 3: Resistance pattern of gram negative uropathogens

Antibiotic name	**Number**	**%R**
Cefuroxime	363	87.6
Amoxicillin/Clavulanic acid	362	83.7
Trimethoprim/Sulfamethoxazole	391	72.9
Ciprofloxacin	389	72.8
Nalidixic acid	362	67.4
Ceftriaxone	377	64.7
Piperacillin/Tazobactam	361	49
Gentamicin	388	42
Cefepime	383	40.2
Nitrofurantoin	373	37.8
Meropenem	389	29.3
Imipenem	382	27
Amikacin	390	22.8
Colistin	379	4
Tigecycline	181	0

Table 4: Resistance pattern of gram positive uropathogens

Antibiotic name	**Number**	R%
Clindamycin	12	83.3
Ciprofloxacin	41	82.9
Levofloxacin	42	65.3
Trimethoprim/Sulfamethoxazole	12	66.7
Rifampin	12	58.3
Vancomycin	42	9.5
Teicoplanin	36	8.3
Linezolid	38	2.6
Daptomycin	21	0
Tigecycline	17	0

The mostly occurring uropathogen showed the antibiotic resistance pattern in the following way where *Escherichia coli* showed 92.9% resistance to Nalidixic acid followed by Ampicillin (85.3%) Ciprofloxacin (78.9%), Trimethoprim/Sulfamethoxazole (71.6%) Cefuroxime (62.8%), Ceftriaxone (61.3%), Cefepime (32.7%), Gentamicin (32.3%) Piperacillin/Tazobactam (23.8%) Nitrofurantoin (12%) Meropenem (11.9%), Imipenem (10.1%) Amikacin (9.6%) and Colistin (0.5%) (Table 5).

It was found that *Klebsiellapneumoniae* showed 96% resitence to Ampicillin followed by Cefuroxime Cefoperazone (73.5%), Trimethoprim/Sulfamethoxazole (70%), Ceftriaxone (68.7%) Nitrofurantoin (67%) Nalidixicacid (667%) Ciprofloxacin (64%) Amoxicillin / Clavulanic acid and Piperacillin/Tazobactam(56%), Gentamicin (53.5%) Meropenem (50%) Cefepime (48.5%) Imipenem (46%) Amikacinand Colistin (1%).

Pseudomonas aeruginosa showed 100% resistance to Ampicillin, Nalidixicacid, Trimethoprim/Sulfamethoxazole, Ampicillin/Sulbactam, Ceftriaxone, Nitrofurantoin, Amoxicillin/Clavulanic acid and Cefuroxime followed by Levofloxacin (80%), Cefoperazone (69.2%), Ciprofloxacin (66.7%), Meropenem and gentamicin (54.2%), Imipenem (50%), Pipercillin/ Tazobactam, Cefepime, Amikacin (45.8%), Doripenem (44.4%), Minocycline (42.9%), Ceftazidime (36.4 %) Aztreonam (33.3 %) Colistin (4.2%). All the isolates of *Escherichia coli* and *Klebsiella pneumonia* and *Pseudomonas aeruginosa* were sensitive to Tigecycline.

Acinetobacter baumannii showed 100% resistance to Nitrofurantoin followed by imipenem (93.8%). It showed 88.2% of resistivity was towards Cefuroxime, Amoxicillin/Clavulanic acid, Ceftriaxone, Meropeneme, Nalidixic acid, Ciprofloxacin and Trimethoprim/Sulfamethoxazole. Moreover, 87.5 % of resistivity was shown towards Ampicillin and Cefepime followed by Gentamicin (82.4%) and Amikacin (64.7%).

Table 5: Antibiotic Resistence pattern of mostly isolated uropathogen

	Escherichia coli		***Klebsiella pneumoniae***		***Pseudomonas aeruginosa***		***Acinetobacter baumanii***	
Antibiotic name	**Number**	**%R**	**Number**	**%R**	**Number**	**%R**	**Number**	**%R**
Nalidixic acid	210	92.9	100	66.7	9	100	17	88.2
Ampicillin	217	85.3	100	96	9	100	16	87.5
Ciprofloxacin	218	78.9	100	64	24	66.7	17	88.2
Trimethoprim/Sulfamethoxazole	218	71.6	100	70	24	100	17	88.2
Cefuroxime	207	62.8	98	73.5	11	100	17	88.2
Ceftriaxone	217	61.3	99	68.7	13	100	17	88.2
Cefepime	214	32.7	99	48.5	24	45.8	16	87.5
Gentamicin	217	32.3	99	53.5	24	54.2	17	82.4
Piperacillin/Tazobactam	202	23.8	91	56	24	45.8		
Nitrofurantoin	216	12	100	67	9	100	17	100
Meropenem	218	11.9	100	50	24	54.2	17	88.2
Imipenem	218	10.1	100	46	24	50	16	93.8
Amikacin	218	9.6	100	39	24	45.8	17	64.7
Colistin	209	0.5	100	1	24	4.2	17	0
Tigecycline	123	0	21	0	10	0	7	0

All the isolates of *Candida albicans* showed sensitivity towards 5-Fluorocytosine, Fluconazole Amphotericin B, Caspofungin and Voriconazole (Table 6).

Table 6: Resistance pattern of *Candida albicans*

Antibiotic name	Number	S%
Amphotericin B	9	100
Caspofungin	9	100
5-Fluorocytosine	9	100
Fluconazole	9	100
Voriconazole	6	100

Distribution of ESBL pathogens and MRSA

Out of the 457 uropathogens isolated, 152 isolates were found to be ESBL producers showing the prevalence by 33.26%. Among all the ESBL isolates highest number of ESBL producer was found to be *Escherichia coli* (66%) followed by *Klebsiella pneumonia* (37.72%) and *Citrobacter freundii* (12.5%). And among the 4 isolates of *Staphylococcus aureus* isolated from urinary tract infected patient the number of MRSA was found to be 3 showing the prevalence rate of 75% (Table 7 and 8).

Table 7: Distribution of ESBL pathogens among uropathogen

S. No.	Organisms	No. of isolates	No. of ESBL producers	Percentage Of ESBL producers
1.	*Klebsiella pneumoniae*	220	83	37.72
2.	*Escherichia coli*	100	66	66
3.	*Citrobacter freundii*	24	3	12.5
	Total ESBL pathogens		**152**	

Table 8: Methicillin Resistant Staphylococcus aureus (MRSA) isolates among uropathogen

Total no. of *Staphylococcus aureus* isolates	No. of MRSA isolated	Percentage
4	3	75%

Figure 1: Resistance pattern of *Escherichia coli*

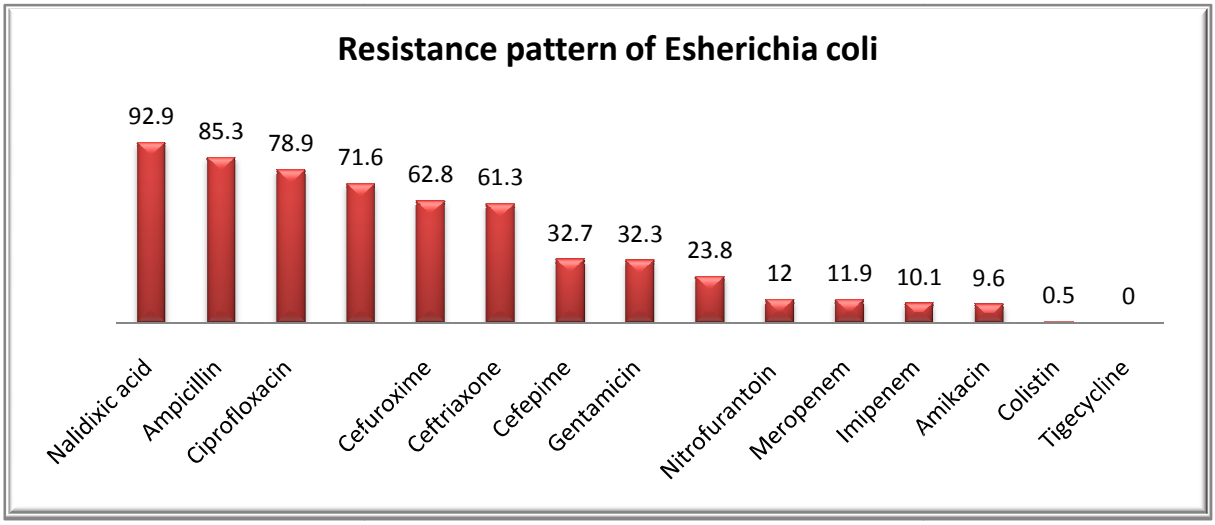


Figure 2: Resistance pattern of *Klebsiella pneumoniae*

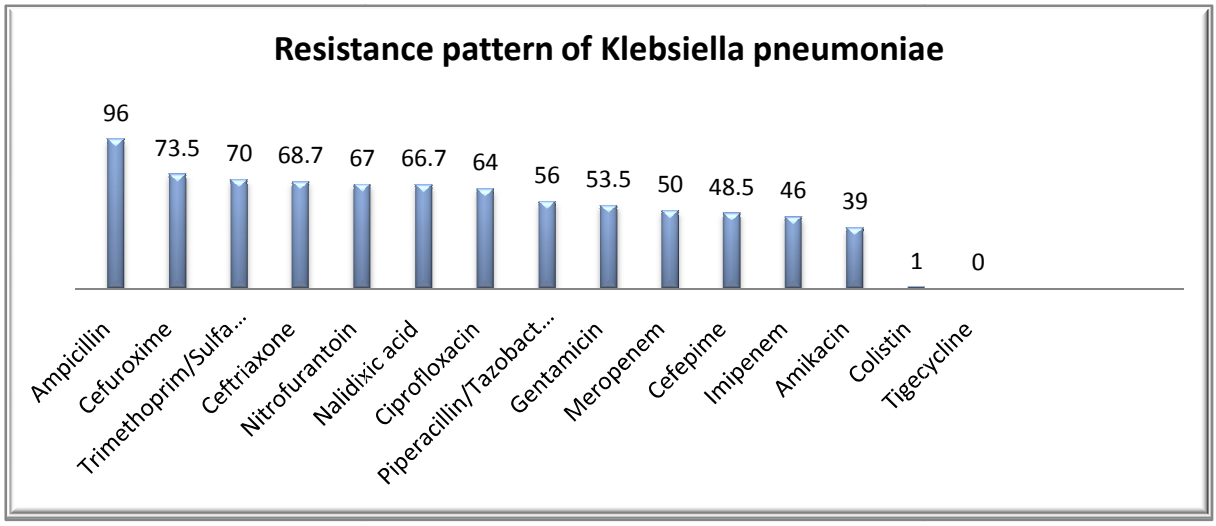


Figure 3: Resistance pattern of *Pseudomonas aeruginosa*

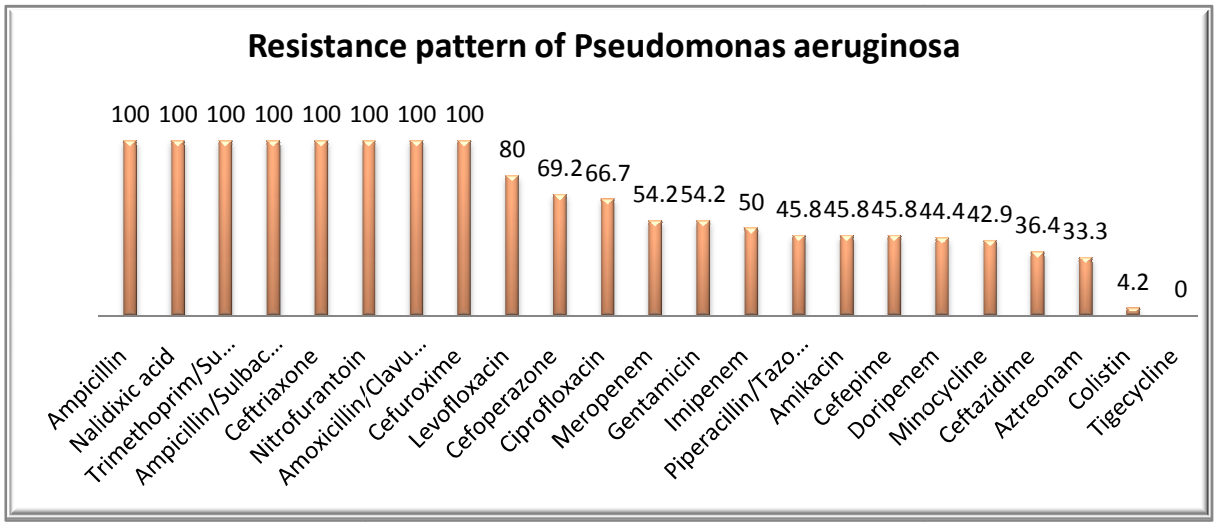
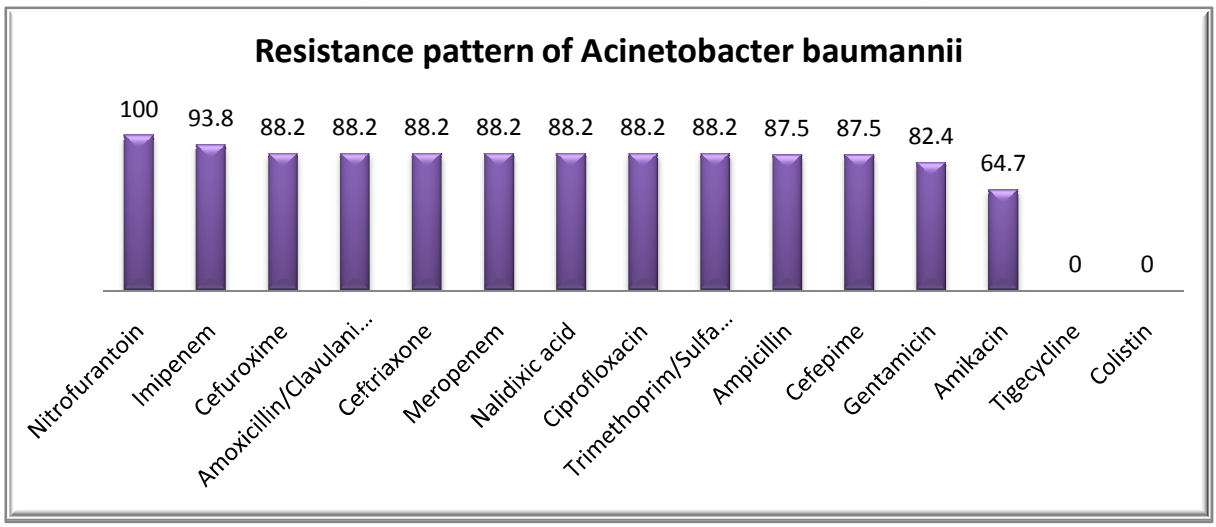


Figure 4: Resistance pattern of *Acinetobacter baumannii*



DISCUSSION

Urinary tract infection is among the most prevalent infectious disease in general population. The effectiveness of an antibiotic administered to a patient depends on the site and severity of the infection, liver and renal function, presence of implants and local resistance patterns. It is also believed that age and pregnancy in the patient determine the effectiveness of the antibiotic used[1]. Recently, with increased rates of antimicrobial resistance, treatment of complicated UTIs has become increasingly challenging for clinicians.

Amoxycillin (a β-lactam antibiotic) was traditionally used in the first line therapy for UTIs, but with the spread of drug resistance, treatment options have now changed. Complicated cases of UTI usually require a longer course or intravenous antibiotics, and in case symptoms do not improve in two or three days, further diagnostic testing is needed. Since bacterial resistance to antibiotics represents a serious problem for clinicians and pharmaceutical industry, efforts have been made recently to reverse this trend by exploring alternate methods[6,29].

For the current investigation, a total of 2090 urine specimens received and processed at Tertiary Health Care centre, located in North Bengal were studied. More than 10^5 colony forming units (cfu) of bacteria/mL of urine were considered significant bacteraemia. Gram-negative isolates were identified up to species level by VITEK 2 automated microbiology system. Of the total 457 isolates, the most commonly isolated bacteria were *Escherichia coli* (48%). Other isolates included *Klebsiella pneumonia* 100 (22%), *Pseudomonas aeruginosa* 24 (5%) and *Acinetobacter baumannii* 20 (4%). Similar kinds of studies have been reported from different regions of India and from other countries have reported that the most prevalent UTI pathogen was *E. coli*, followed by *Klebsiella* spp[12,26,20,28]. 116 uropathogens was isolated from critical unit and 141 from semi critical. Data analysis revealed that urinary tract infection was more prevalent in women (59%) to be as compared to men (41%) similar to the study of Dugal et al.,[11]. It is known that UTI occur more commonly in women, with half of them having at least one infection at some point in their lives. It is believed that bacteria are usually transmitted to the urethra from bowel, with females at greater risk due to their anatomy. During pregnancy, high progesterone levels elevate the risk of decreased muscle tone of the ureter and bladder, which leads to a greater likelihood of reflux, towards the kidneys[11].

For the study, the antibiogram pattern of the 457 isolates was checked against 16 antibiotics belonging to different groups and possessing varied modes of action.

Escherichia coli showed highest resistance against nalidixic acid (92.9%), ampicillin (85.3%) , followed by Ciprofloxacin (78.9%) similar to the result of Ahmed et al 2014 who found similar type of resistance pattern for *Escherichia coli* that is nalidixic acid (98.5%) and ciprofloxacin (86.2%).

It was reported that *Klebsiella* spp. from Eastern India UTI samples were maximally resistant to penicillin combination, followed by aminoglycosides and third generation cephalosporin. Studies conducted in West Bengal and around other parts of the country showed consistency in *Klebsiella pneumonia* which presented the second highest resistance after *E. coli*, against different classes of antibiotics[18]. Data are consistent with the findings of a northern Indian city where *Klebsiella pneumonia* showed the highest resistance to a drug from the penicillin combination similar to the present study where *Klebsiella pneumonia* showed 96% resistance to a drug from penicillin combination followed by third generation cephalosporin, cefuroxime and cefoperazone.

Data analysis showed that all *Pseudomonas aeruginosa* isolate showed 100% resistance to Ampicillin, Nalidixic acid, Trimethoprim/Sulfamethoxazole, Ampicillin/Sulbactam, Ceftriaxone, Nitrofurantoin, Amoxicillin/Clavulanic acid and Cefuroxime. *Acinetobacter baumannii* isolates showed high level of resistence to nitrofurantoin followed by imipenem.

A high level of sensitivity was noted to colistin and tigegcycline by all gram negative bacilli.

The accurate detection of extended-spectrum β-lactamases is a major clinical problem, particularly in invasive infections, frequently leading to therapeutic failure and adverse clinical outcome. In typical circumstances, ESBLs derive from genes for TEM-1, TEM-2, or SHV-1 by mutations that alter the amino acid configuration around the active site of these β-lactamases.

This extends the spectrum of β-lactam antibiotics susceptible to hydrolysis by these enzymes. Successful spread of ESBL-encoding genes within the microbial genome can be attributed to their common localization on self-transmissible or easily movable broad-range plasmid[23].

The Advanced Expert System (AES) in conjunction with the VITEK 2 automated antimicrobial susceptibility test system is widely used in clinical microbiology laboratories for the identification and evaluation of the susceptibility profiles of bacteria and helps in the detection of extended-spectrum β-lactamases (ESBLs) produced by organisms.

The phenotypic data generated in the current study, using this system, indicates a considerably significant prevalence of ESBL producers in the region of North bengal, where a total of 152 out of 457 (33.26%) uropathogens were found to be ESBL producers which was very similar to the study conducted in the Central Referral Hospital, Gangtok where the prevalence of ESBL was found to be 34.03%[30]. On the contrary 27.67% uropathogens were found to be ESBL producers by Dugal *et al.*,[11] in a similar kind of study conducted at Mumbai hospital.

The identification of the *mec*A gene is the most reliable method for detecting the MRSA isolates. However, not all laboratories can include molecular biology techniques in their routine clinical practice. So, it is important that phenotypic techniques which are able to detect the MRSA isolates in a rapid and accurate manner are made available, in order to ensure the correct antibiotic treatment and to avoid the spread of the MRSA isolates in the hospital environment[9].

The prevalence of MRSA in our study was 75% while Dalela *et al.*,[9] in Jhalawar found prevalence rate as 42.4%. Sanjana R K *et* al.,[28] in Nepal, detected the prevalence of MRSA as 39.6%, Rajaduraipandi K *et al*.,[27] in Coimbatore found 31.1% strains of MRSA and Anupurba S *et al.*,[4] in eastern Uttar Pradesh found a 54.85% prevalence of MRSA, which correlated well with the findings of our study. Onanuga A *et al*.,[22] in Nigeria have reported a high prevalence of 69%, while Coombs G W et al.,[8] in Australia found it to be very low as 16%.

CONCLUSION

Despite the advances in diagnostic methods, availability of antimicrobials and awareness among the people, urinary tract infections continue to remain a major health problem and the resistance pattern of multi drug resistant uropathogen is in rise. In the current study, among the oral drugs, broad spectrum penicillin, amoxycillin/clavulanic acid, quinolone drugs, and third generation cephalosporins like ceftriaxone and cefuroxime and should no longer be considered as the first line drugs for the empirical treatment of clinically evident UTI, because of the very high resistance rates. Moreover, a significantly higher number of ESBL and MRSA were found.

However, the present investigation was carried in a particular healthcare centre, additional studies can be carried out with a larger sample size from various hospitals in the region to obtain a more representative picture. Moreover, control measures which include the judicious use of antibiotics, antibiotic cycling, and the implementation of appropriate infection control measures and the formulation of an antibiotic policy must be done, to prevent the spread of these strains.

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