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Uropathogens and the Common Resistance Mechanisms using Vitek 2 System: A Retrospective Study in North Goa District Hospital

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Abstract: ***This study was performed to determine the prevalence of various pathogens in cases of urinary tract infection at North Goa district hospital, to study the resistance pattern and resistant phenotypes of the organisms. Study was conducted in the microbiology laboratory on 662 urine samples from inpatient and outpatient departments of North Goa district hospital. Fully automated Vitek 2 compact System was used in identification and antimicrobial susceptibility testing of the causative organism. Of 662 samples received, 68 urine samples (10.2%) were found to show growth of pathogens responsible for UTI. Most frequently isolated bacteria were Escherichia coli (70%), Klebsiella pneumoniae (17.5%), Proteus species (5%), Enterobacter cloacae (5%), Pseudomonas aeruginosa (2.5%). Antibiotic resistance was commonly observed in ampicillin (80.8%), ticarcillin (75.8%) cefalotin (68.2%), ceftriaxone (57.4%), ciprofloxacin 54%), amoxy clav (48.5%), levofloxacin (42.7%).52.9% of the isolates were ESBL producers. Phenotypic expression of 2 to 3 types of resistance mechanisms were recorded in 42% of the isolates. CTX - M type was the most common type of ESBL expressed. The study shows that automated system like VITEK 2 helps in early reporting and detection of resistance mechanisms with proper identification of organisms causing UTI and stresses on reducing the emergence of antibiotic resistance.***

Keywords: ESBL, VITEK 2, UTI

1. Introduction

UTI is a common health issue in the population especially in women. Around 50 - 60%women suffer from UTI at least once in their life ([5]. Recurring infections can occur if predisposing conditions are not identified and antibiotic therapy is given without observing the resistance pattern in each case. Broad spectrum antibiotic usage for syndromic management has led to increase in resistance in bacteria. The antibiotic resistance pattern varies from country to country ([1], also from region to region based on various factors. Hence regional studies should be conducted to monitor changes in susceptibility of common pathogens periodically. Antibiotics should be started after culture report specific to the infecting organism.

2016 guidelines by NCDC recommend nitrofurantoin, ciprofloxacin or cotrimoxazole as empiric therapy before culture is available. Flouroquinolones are starting to show increasing resistance and thus there has been increase in use of older agents like nitrofurantoin and fosfomycin. [7]

Fosfomycin is a single dose therapy and increased resistance is documented in regions with frequent use. It has a wide spectrum of activity and a favourable safety profile, but it's continued oral administration at an insufficient dose, may rapidly diminish usefulness, especially for iv administration [6]. Nitofurantoin has a favourable microbiological as well as clinical outcome, as compared to other agents.

Fully automated VITEK 2 system allows for identification of patterns of resistance and the mechanisms, in addition to recording individual susceptibility results. The MIC phenotypes found for the isolate, is compared to the patterns in the database and the best match is identified. [7]

Beta - lactam antibiotics are commonly used to treat infections with gram negative bacteria. Although beta lactams are not commonly used for treating UTI, newer agents like cephalosporins are prescribed especially in pregnancy. Flouroquinolones which are first line agents for treatment of UTI are not safe to use in pregnancy, and sulfonamides are not safe in third trimester. The persistent exposure of bacterial strains to a multitude of β - lactams has induced dynamic and continuous production and mutation of β - lactamases in these bacteria, expanding their activity even against the newly developed β - lactam antibiotics. [9]

Extended - spectrum beta - lactamases (ESBL) are enzymes that confer resistance to most beta - lactam antibiotics, including penicillins, cephalosporins, and the monobactam aztreonam but are inhibited by beta lactam inhibitors. ESBLS are plasmid mediated and inactivate a wide variety of beta lactam antibiotics by hydrolyzing the beta lactam ring. ESBLS are classified according to their amino acid sequence, most common being CTX - M type, SHV type, AMP - C. They can be detected phenotypically in clinical laboratories by testing with ceftazidime and cefotaxime in conjunction with clavulunate (3). VITEK 2 detects MIC, analysis the MIC Patterns displayed by the bacteria under study, and then interprets the resistance pattern by the software AES - Advanced Expert System.

Aim of study

- To study the prevalence of various pathogens in cases of urinary tract infection presenting at District Hospital
- To determine the most sensitive oral antibiotics in cases of UTI
- Determine the sensitivity to commonly used oral agents in our hospital
- Study the resistance patterns and resistance phenotypes in our hospital

2. Material and Methods

This study was conducted in microbiology laboratory of pathology department of North Goa District Hospital. Retrospective analysis was performed on 662 urine samples received in the laboratory from OPD and IPD of North District Hospital from July 2020 to July 2021 and antibiotic susceptibility test results were evaluated. Patients were instructed regarding proper collection of urine sample. Clean catch midstream urine was cultured on mackonkey agar and blood agar by semi quantitative technique using 2 mm disposable plastic loop. All samples showing more than 10, 000 CFU/ml of pure growth were studied and evaluated using Vitek 2 automated ID and AST system. Standard procedure was followed to inoculate the growth into the specific antibiotic sensitivity cards. Sensitivity to individual antibiotics was recorded as minimum inhibitory concentration (MIC). Also any resistance phenotype recorded by AES system was noted. Results were automatically modified by the system according to the resistance phenotype identified.

Antibiotic resistance was assessed across age groups. Antibiotics assessed were beta lactams (ampicillin, amoxycillin clavulunate, piperacillin tazobactam, cefixime, ceftriaxone, ceftazidime), Aminoglycosides (gentamicin, amikacin), cotrimoxazole, nitrofurantoin, fosfomycin (only for Escherichia coli) and flouroquinolones (ciprofloxacin, norfloxacin, ofloxacin). Intermediate results were considered to be sensitive, as these agents are known to be concentrated in urine.

3. Results

662 samples were received over a period of 1 year, out of which 68 urine samples showed growth of pathogens.

163 samples showed a growth of 2 or more pathogens, due to improper collection.82% of these samples were from females. Recollection of samples was requested in these cases.

Most frequently isolated bacteria were Escherichia coli (70% of isolates), Klebsiella pneumoniae (17.5%), Proteus species (5%), Enterobacter species (5%) and Pseudomonas aeruginosa (2.5%)

Among the patients showing UTI, 75% were females and 25 % were males.

17.5% patients were less than 18 years, 47.5 % were 18 to 45 years and 35% were more than 45 years

Bacteriological proven UTI was seen in 10.2% of suspected urinary tract infection.

Table 1: Distribution of Organisms among Culture Positive Samples

	Total (n=68)		Males (n=17)		Females (n=51)	
Escherichia coli	47	69.1%	10	58.8%	37	72.5%
Klebsiella *sp*	12	17.6%	5	29.4%	7	13.7%
Proteus *sp*	4	5.8%	0	-	4	7.8%
Enterobacter *sp*	3	4.4%	0	-	3	5.8%
Pseudomonas aeruginosa	2	2.9%	2	11.7%	0	5.8%

All the culture positive samples in our study, showed growth of gram negative bacilli. No UTI caused by gram positive cocci or candida was seen. Escherichia coli was the most common causative agent (69%), followed by Klebsiella species (17.6%), Proteus species (5.8%), Enterobacter species (4.4%), and Pseudomonas aeruginosa (2.9%). Among females, 72% of infections were caused by E. coli, 13.7% by Klebsiella *sp* and rest by Proteus *sp*, Enterobacter *sp* and Pseudomonas aeruginosa (7.8%, 5.8%, 5.8% respectively) whereas in males, 58.8% infections were caused by Escherichia coli, 29.4% by Klebsiella *sp* and 11.7% by Pseudomonas aeruginosa.

Table 2: Antibiotic Susceptibility Percentage of the Uropathogens Isolated From Culture Positive Samples

Organism	Total number of isolates	Cotrimoxazole	Nitrofurantoin	Fosfomycin	Ciprofloxacin	Levofloxacin	Ampicillin	Amoxy - clav	Ticarcillin	Pipercillin - Tazobactam	Cefalotin	Cefoxitin	Ceftriaxone	Ceftazidime	Ertapenem	Amikacin	Genatamicin
Escherichia coli	47	35 (74.4)	42 (89.3)	47 (100)	18 (38)	22 (46)	10 (21)	23 (49)	12 (25.5)	40 (85)	12 (25.5)	30 (64)	18 (38)	23 (49)	45 (95.7)	47 (100)	40 (85)
Klebsiella *sp*	12	12 (100)	7 (58)	-	7 (58)	10 (83)	-	7 (58)	3 (25)	10 (83)	7 (58)	8 (66.6)	7 (58)	10 (83)	12 (100)	12 (100)	12 (100)
Proteus *sp*	4	4 (100)	0	0	0	2 (50)	0	4 (100)	0	4 (100)	0	0	0	2 (50)	4 (100)	4 (100)	2 (50)
Pseudomonas aeruginosa	2	NA	NA	NA	2 (100)	2 (100)	-	NA	NA	2 (100)	NA	NA	2 (100)	2 (100)	NA	2 (100)	2 (100)
Enterobacter *sp*	3	3 (100)	3 (100)	NA	3 (100)	3 (100)	-	0	1 (33.3)	3 (33.3)	2 (66.6)	0	2 (66.6)	3 (100)	3 (100)	3 (100)	3 (100)

*Figures in brackets denote percentage of isolates, susceptible to the antibiotic

Among Escherichia coli isolates, fosfomycin was 100% sensitive, followed by nitrofurantoin (89%) and cotrimoxazole (74%)

Table 3: Overall Resistance Pattern Seen in Gram Negative Bacilli

Antibiotic	% Sensitivity	% Resistance
Cotrimoxazole	81%	19%
Nitrofurantoin	78%	22%
Fosfomycin	100%	0
Ciprofloxacin	44%	56%
Levofloxacin	57.3%	42. .7%
Ampicillin	19.2%	80.8%
Amoxy - Clav	51.5%	48.5%
Ticarcillin	24.2%	75.8%
Piperacillin Tazobactam	86.7%	13.3%
Cefalotin (first generation cephalosporin)	31.8%	68.2%
Cefoxitin (second generation cephalosporin)	57.5%	42.5%
Ceftriaxone (Third generation cephalosporin)	42.6%	57.4%
Ceftazidime (Third generation cephalosporin)	58.8%	41.2%
Ertapenem	96.9%	3.1%
Amikacin	100%	0
Gentamicin	86.7%	13.3%

Table 4: Mechanism of Resistance detecetd by Vitek 2 System

Resistance mechanism	No of Gram negative bacilli showing resistance mechanism	Percentage prevalence of resistance mechanism
ESBL	36	52.9%
ESBL (CTX - M type)	5	7.3%
ESBL (AMPC Type)	2	2.9%
ESBL (SHV Type)	2	2.9%
Penicillinase	13	19.11%
Wild Phenotype (No Resistance)	10	14.7%

52.9% of the isolates were ESBL producers. Phenotypic expression of 2 to 3 types of resistance mechanisms were recorded in 42% of the isolates. CTX - M type was the most common type of ESBL expressed.

4. Discussion

In our study 10.2% percent of the clinically suspected UTI were confirmed microbiologically. Symptoms like frequency of micturition, abdominal pain, fever may not be enough to diagnose UTI. Other factors like presence of significant amount of pus cells in urine, past history of UTI, need to be considered to diagnose UTI. The patients could be also receiving empiric therapy before culture is done. This could result in no growth in the culture, and recurrence of infection after some time, in case the prescribed antibiotic was resistant. This implies that culture is an essential investigation to diagnose UTI, and prevent unnecessary prescription of antibiotics. Studies conducted in India have shown similar positivity rate. ([11]) However some of the studies showed higher positivity 37% Christy et al ([14]) and 34.2% by Prakashan et al ([16]) as these studies included patients with high risk factors like diabetes and nephrolithiasis.

UTI is a contagion among both men and women, but incidence is found to be higher in women due to biological factors like short length of urethra and proximity to anal opening. ([4]) Escherichia coli UTI was seen in greater proportion in females (72% of all UTI in females) compared to 58% of all UTI in men. This could because of the proximity of urethra to anal orifice in females and improper hygiene, sexual activity could increase the colonistion by coliform bacteria.

Escherichia coli comprised of 69% of all UTI, followed by Klebsiella species (17.6%), Proteus species (5.8%), Enterobacter species (4.4%), and pseudomonas (2.9%). In a study by Chandrashekhar D et al, Escherichia coli was the major isolate followed by Klebsiella, Citrobacter, Enterococcus, Enterobacter, Pseudomonas, coagulase - negative Staphylococcus, and Proteus. ([15], [16])

Antimicrobial resistance is increasing worldwide resulting in infections that are difficult to treat. This has resulted in higher mortality and morbidity. The high incidence of UTI and tendency to recur leads to high healthcare cost.

Among the gram negative bacteria isolated, resistance to commonly used antibiotics like Nitrofurantoin was low at 22%. Other studies in India have seen 28% and 35% resistance. ([10], [13]) Nandini et al found 50% resitance to nitrofurantoin. Chaudhary et al ([17]) showed 20% resistance in E coli and around 60 - 70% in other isolates like Klebsiella and citrobacter. Nitrofurantoin is a good option for treatment of UTI as it is concentrated in urine and shows less resistance in our hospital.

Resistance was high for ciprofloxacin 56% and levofloxacin 42%. Similar level of resistance was seen in Ciprofloxacin in other studies in India ([10], [13]) 74% resistance reported by Eswarappa et al, 66 % by prakasam et al, 50% by Choudhary et al and 65% by Kothari et al. Nandini et al obtained 52% sensitivity to ciprofloxacin, compared to 25% to levofloxacin. Treatment with flouroquinolones is not advisable without a culture report, especially as empiric therapy. Levofloxacin showed a better chance of being effective in our study also. Increasing level of resistance is being seen for this antibiotic all over India and the world.

Cotrimoxazole showed low resistance 19%, findings contradicting to some studies ([13]) and consistent with other study. ([11]) Prakasam et al reported 100% sensitivity to Cotrimoxazole (16).15% sensitivity in Ecoli and 50% sensitivity in Klebsiella was seen by Nandini et al. In contrast, Sensitivity to cotrimoxazole was 74 % in Ecoli and 100 % in Klebsiella in our study. Fosfomycin is an older antibiotic which is being used in recent times, due to emerging resistance to commonly used antibiotics. No resistance was observed, but it should be used cautiously, in order to prevent emergence of resistance.

In study by Nandini et al, the maximum sensitivity of E coli was found to the aminoglycosides (72 - 76%), cefoperazone - sulbactam (72%) nitrofurantoin (52%), carbapenems (33 - 38%) and piperacillin tazobactam (33%). They were mostly resistant to all quinolones except levofloxacin (52%) and all cephalosporins (except sulbactam combinations).

High level of ESBL producing gram negative bacteria were found (52.9%) in our institution. This raises concerns over use of Beta Lactams especially single agents like amoxicillin especially in pregnant patients where these maybe the drug of choice. Combination of Beta lactams and Beta Lactamase inhibitors are likely to be more effective. Study by Faryabi et al, showed ESBl Escheria coli as the most common organism and suggested dual drug regimens to be more effective outcomes. [(14)] Among the bacteria isolated by Nandini et al (19), 23.2% were ESBLs in E. coli and 9.4% were ESBLs in K. pneumoniae. ESBL expression is found to increasing in recent years. In a study from Jaipur, ESBL producing E. coli (from 9.52% to 30.08%) has been noticed over a period from 2007 - 2009. (20)

In a study by Dallela et al, the prevalence of ESBL and ampC β - lactamase and the coexistence of these phenotype (ESBL + ampC β - lactamase) in the urinary isolates was found to be 66.9%, 21.1%, and 3.5% respectively (22). They also observed co presence of ESBL and AmpC Beta lactamase in 3.5% of the isolates. In our study, 2 (2.9%) isolates showed expression of both these enzymes. In a study by Fennell et al, E. coli predominated (90.9%) followed by K. pneumoniae (5.6%) among organisms producing ESBLs.

Detection of resistance is of significance for epidemiological and infection control purposes. Among Beta Lactams, carpenems are most effective against ESBLs [(23).] Although ESBL activity is inhibited by clavulanic acid, the only infections that may be treated safely with b lactam/b - lactamase inhibitor combination are those involving the urinary tract. In this instance, b - lactamase inhibitor concentration is high enough to counteract the hydrolytic activity of ESBLs ([23])

24.6% of samples were reported as contaminated and required recollection of samples among which, 82% of samples were from females. Sample collection procedure needs to be reinforced among nursing staff who may be instructing the patients regarding the same. Patients need to clean perineal area properly with soap and water, and collect clean catch midstream sample. Proper history of symptoms should be provided on requisition, so that the significance of isolated organisms can be better predicted.

5. Conclusion

Antibiotic resistance is a major threat to human health and there is a need of conserving and stewarding the effectiveness of antimicrobials used for treatment. The way of reducing the emergence and spread of antibiotic resistance is to introduce more diversity into antibiotic prescribing and stress on evidence based management, and not use antibiotics irrationally. Empiric treatment for UTI with broad spectrum antibiotic should not be done before culture, as it alters the results, and may lead to incomplete and improper treatment. Automated system like VITEK 2 offer the advantage of early reporting (34 hours), helps in detection of resistance mechanisms and proper identification of isolated organism which in turn affects the reporting of susceptibility results. Also stress need to be placed on proper sample collection, as improper collection may give inconclusive results due to contamination which may cause delay in the diagnosis and initiation of therapy.

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