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INCIDENCE OF ANTIBIOTIC RESISTANCE OF *ESCHERICHIA COLI* FROM PATIENTS WITH SUSPECTED URINARY TRACT INFECTION IN CRITICAL CARE UNIT AT TERTIARY CARE HOSPITAL, KOLKATA.

Microbiology

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ABSTRACT

BACKGROUND: Antimicrobial resistance is one of the most important threats to human health. Multiple surveillance programs have been launched worldwide to monitor the spread of resistant strains in community acquired and noncosmial infections. Urinary tract infection (UTI) is the second most common bacterial infection managed in primary care and *Escherichia coli* is the most common pathogen causing UTI.[5,6] Selection of empiric antibiotics for urinary tract infections (UTI) has become more challenging because of the increasing rates of multidrug resistant organisms especially multidrug resistant enterobacteriaecae(MDRE) mostly ESBL's and carbapenemases .[(1)] Enterobacteriaecae are the most common cause of urinary tract infections (UTI) in both community and healthcare settings. Selection of empiric antibiotics for UTI's is often therefore based on the local or institutions susceptibility profiles.

MATERIALS AND METHODS:- The study was conducted among the different age groups of both males and females attending at KPC Medical College, Jadavpur, Kolkata during two month of period included in this study. A total of 100 urine samples received from patients of the ICU of the medical college & hospital. All urine samples were processed within 1 hour after collection for aerobic bacterial culture.

RESULTS:-

1. Bacterial isolate specimens and population characteristics:- A total of 50 *E. coli* isolates were obtained from the collected urine samples, of which 19 isolates were derived from men and 31 *E. coli* isolates from women.

Forty –seven (94%) of the 50 *E. coli* isolates were resistant to at least one of the tested antimicrobial agents. Thirty-five (70%) of the 50 *E.coli* isolates were resistant to more than one antimicrobial agent.

Out of 100 urine specimens processed in this study, 75(75%) showed significant bacteriuria.

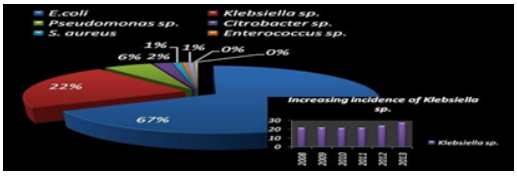
This study was done in the period June 2018 to August 2018 in KPC Medical College &hospital.

CONCLUSION:- Urine infection is one of the most common medical problems affecting the population. This retrospective study was performed with the aim of surveying the incidence of antibiotic resistance patterns of bacterial agents, isolated from patients with UTI-positive cultures between June 2018 and august 2018 in KPC medical college & hospital Kolkata. In the present study, 75 patients with UTI-positive cultures were identified, 50 cases (66.6%) were female and 25 cases (33.3%) were male.

KEYWORDS

Urinary tract infections (UTI), Antimicrobial resistance, Significant Bacteriuria, Escherichia coli, Enterobacteriaecae, multidrug resistant enterobacteriaecae(MDRE).

INTRODUCTION



Bacterial urinary tract infection (UTI) becomes the most common type of infection affecting the urinary tract and causing inflammation of bladder and kidneys. Most of the clinicians prescribe antibiotics to treat UTI infections, resulting in failure of treatment in many cases due to occurrence of bacterial drug resistance. The purpose of this study to know the bacterial causative agents causing UTI, antimicrobial susceptibility pattern of isolates of different age group of persons from KPC medical college and hospital, jadavpur, west Bengal, India.

The present study approaches the following objectives: -

- Isolation, identification and biochemical characterization of urinary tract infection causing bacteria.
- Incidence of UTI among various patients on the basis of their sex.
- To determine antibiotic susceptibility of E. coli prevalent in community acquired infection.

MATERIALS AND METHODS:-

The study was conducted among the different age groups of both males and females attending at KPC Medical College, Jadavpur, Kolkata during two month of period included in this study. A total of 100 urine samples received from patients of the ICU of the medical college & hospital. All urine samples were processed within 1 hour after collection for aerobic bacterial culture.

Materials required:-

Glassware and other apparatus:-

- Spirit lamp
- Inoculating loop
- Laminar air flow
- Forceps
- Millimeter ruler
- Alcohol
- Cartridges of antibiotic discs
- Sterile petri plates
- 37°C Incubator

Media used for culture:

1. UTI Agar

Hi Crome UTI Agar is a differential medium recommended for presumptive identification of microorganisms mainly causing urinary tract infections.

2. Mac conkey agar

Mac Conkey Agar is recommended for selective isolation of *Escherichia coli* from pharmaceutical products. It is also recommended for selective isolation and differentiation of lactose fermenting and lactose non fermenting enteric bacteria.

3. Mueller Hinton Agar for antibiotic sensitivity

Recommended for determination of susceptibility of microorganisms to antimicrobial agents isolated from clinical samples.

Specimen collection:-

Specimens of urine are collected in plastic universal containers, but mid – stream specimens from females are more conveniently collected in a wide- mouthed container such as 12 oz. (350 ml) glass jar or a sterile waxed cardboard container. From male patients, *a mid-stream specimen of urine* (MSU, the middle of the urine flow) was collected.

Transport of specimen

Once collected, a specimen of urine was transported to the laboratory without delay, for urine is an excellent culture medium and contaminating bacteria can readily multiply to reach apparently

significant numbers.

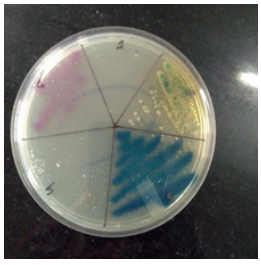
METHODOLOGY:-

Microscopy of urine:-

Microscopical examination of urine was done principally to detect the presence of increased numbers of polymorphs (pyuria) as an indication of infection in the urinary tract when culture may fail to show significant bacteriuria.

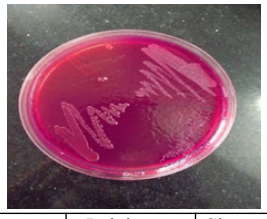
Wet film examination:-

A leucocyte count sufficiently accurate for general purposes may be obtained from examination of a wet film of uncentrifuged urine, provided that the area of the microscope field is known and the depth of the film is standardized.



Standard loop method

An inoculating loop of standard dimensions was used to take up a small, approximately fixed and known volume of mixed uncentrifuged urine and it was spread over a plate of agar culture medium. The plate was incubated, the number of colonies counted or estimated, and this number used to calculate the number of viable bacteria per ml of urine. Thus, if a 0.004 ml loopful of urine yields 400 colonies the count per ml will be 105, or just indicative of significant bacteriuria.

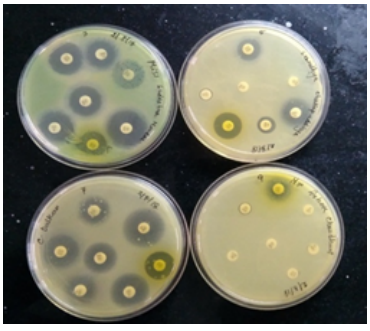


Detection of antibacterial activity in urine

Antibiotic sensitivity describes the susceptibility of bacteria to various antibiotics. It is often done by the Kirby-Bauer methods.

Kirby Bauer Disc Diffusion Method

Three to five identical colonies were picked from an overnight grown primary agar plate with a sterile loop and was suspended in 0.5ml of Peptone water. The turbidity was matched with 0.5 McFarland turbidity standards. A fresh, sterile cotton tipped swab was dipped into this suspension and the excess of inoculum was removed by pressing it against the sides of the tube.



ANTIBIOTIC SUSCEPTIBILITY TESTING DISC DIFFUSION

a) *Psuedomonas aeruginosa* sensitivity testing showing in sample no. 3, sensitive to NX,LE,GEN,IPM,PIT.
b) *Escherichia coli* sensitivity testing showing in sample no. 5, resistant to CXM, NX, LE.
c) *Escherichia coli* sensitivity testing showing in sample no. 7, sensitive to NX,LE,GEN,IPM,PIT,NIT.
d) Multi-drug resistant *Escherichia coli* showing in sample no. 9, resistant to NX, LE, GEN, IPM, PIT, NIT, and CXM.

BIOCHEMICAL REACTIONS FOR IDENTIFICATION OF BACTERIAL ISOLATES[12]

Presence of lactose fermenting large, dry, colonies with irregular margins and lactose fermenting large mucoid colonies on Mac Conkey agar medium confirmed by gram staining. These were then subjected to preliminary tests and biochemical tests:-

Organisms	Indole test	Citrate utilization test	Urease test	Mannitol fermentation test	Triple sugar iron test	Coagulase test
E. coli	Positive (+ve)	Negative (-ve)	Negative (-ve)	Fermented	A/A+/- Gas	-
K. pneumonia	Negative (-ve)	Positive (+ve)	Positive (+ve)	Fermented	A/A+ Gas	-
Pseudomonas aeruginosa	Negative (-ve)	Positive (+ve)	Negative (-ve)	Not-Fermented	K/ NC	Negative (-ve)
Enterococcus faecilis	Negative (-ve)	Negative (-ve)	Negative (-ve)	Fermented	K/ NC	-
S. aureus	Negative (-ve)	Positive (+ve)	Positive (+ve)	Fermented	K/ NC	Positive (+ve)
CONS	Negative (-ve)	Positive (+ve)	Positive (+ve)	Fermented	K/ NC	Negative (-ve)

"K"= Alkaline "A"= Assacharolytic "NC"= No change

RESULTS:-

1. Bacterial isolate specimens and population characteristics:-

A total of 50 *E. coli* isolates were obtained from the collected urine samples, of which 19 isolates were derived from men and 31 *E. coli* isolates from women.

Forty –seven (94%) of the 50 *E. coli* isolates were resistant to at least one of the tested antimicrobial agents. Thirty-five (70%) of the 50 *E.coli* isolates were resistant to more than one antimicrobial agent.

Out of 100 urine specimens processed in this study, 75(75%) showed significant bacteriuria.

This study was done in the period June 2018 to August 2018 in KPC Medical College &hospital. The results of the study are described in the respective tables:-

Table 1: Details of isolates:-

Name of the isolate	No. of isolates	Percentage n= 75
Escherichia coli	50	66.66%
Klebsiella pneumoniae	15	20%
Pseudomonas aeruginosa	6	8%
Enterococcus faecalis	2	2.66%
Staphylococcus aureus	1	1.34%
CONS	1	1.34%

Commonly isolated organisms from urine samples of UTI cases

Table 2: Distribution of isolates with respective group:-

Type of Bacteria Isolated	Total n=75
GPC	4 (5.33%)
GNB	71(94.6%)

2. Isolate antibiotic susceptibility:-

All isolates were analyzed by agar disc diffusion to determine their susceptibility patterns to the 25 tested antimicrobial agents.

Table 3:- Comparison Of Sex Based Antibiotic Resistance Pattern Of E.coli Strains Isolated From Patients With Suspected Urinary Tract Infection:-

Antimicrobial Agents	Male			Female		
	No. of resistance	No. of non -resistance	Rate of resistance (%)	No. of resistance	No. of non –resistance	Rate of resistance(%)
LE5	11	8	57.8	17	14	54.8
NX10	12	7	63.1	20	11	64.5

PIT100/10	4	15	21	14	17	45.1
GEN10	6	13	46.1	8	23	25.8
AK30	8	11	42.1	16	15	51.6
PB300	9	10	47.3	10	21	32.2
NA30	11	8	57.8	20	11	64.5
CPM30	12	7	36.8	16	15	51.6
IPM10	7	12	36.8	9	22	40.4
MRP10	6	13	31.5	10	21	32.2
CIP5	10	9	52.6	13	18	41.9

Antimicrobial Agents	Male			Female		
	No. of resistance	No. of non -resistance	Rate of resistance (%)	No.of resistance	No. of non –resistance	Rate of resistance (%)
NIT300	2	17	10.5	9	22	29
COT25	6	13	31.5	7	24	22.5
NET10	5	14	26.3	3	28	9.6
AMC30	10	9	52.6	12	19	38.7
CL10	7	12	36.8	11	20	35.4
CAZ10	4	15	21.0	15	16	48.3
CFS	9	10	47.3	13	18	41.9
CXM30	15	4	78.9	21	10	67.7
CX30	6	13	31.5	19	12	61.2
E15	9	10	47.3	9	22	40.4
AZ10	8	11	42.1	12	19	38.7
CTX30	5	14	26.3	11	20	35.4
TOB10	2	17	10.5	5	26	16.1
CF30	3	16	15.7	8	23	25.8

Table 4:- Antibiotic resistance patterns of *E. coli* strains

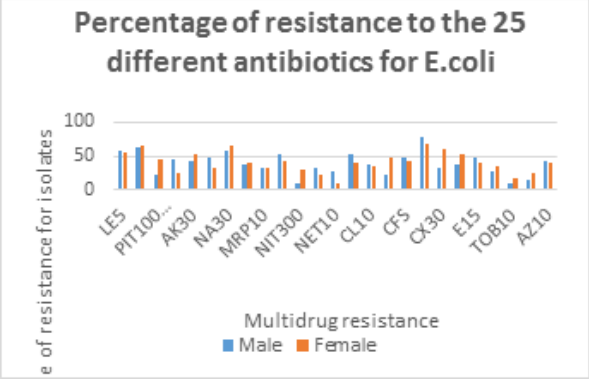
Antibiotic resistance % of isolates

Antimicrobial agents Males	Females
ß-lactams/ß-lactamase inhibitor Combinations	
PIT 100/10	21.0
45.1	
CFS	47.3
41.9	
AMC 100	52.6
38.7	
Cephems	
CX 30	31.5
61.2	
CTX30	26.3
35.4	
Carbapenems	
IPM10	36.8
40.4	
MRP10	31.5
32.2	
Aminoglycosides	
GEN10	46.1
25.8	
TOB10	10.5
16.1	
Ak30	42.6
52.1	
NET10	26.3
9.1	

Antibiotic resistance % of isolates

Antimicrobial agents	Females	Males
Macrolids		
AZ10	42.1	38.7
E15	47.3	40.4
Polymyxins		
CL10	36.8	35.4
PB300	47.3	32.2
Quinolones		
Nx10 63.1	64.5	
CIP5 52.6	41.9	
Le5 57.8	54.8	
Na30 57.8	64.5	
2nd gen Cephalosporins		
CF 30	15.7	25.8
CXM30	78.9	67.7
3rd & 4th gen cephalosporins		
CAZ10	21.0	48.3
CPM30	36.8	51.6
Nitrofuran		
NIT300	10.5	29.0
Trimethoprin-sulfamethoxazole		
COT 25	31.5	22.5

3. Multidrug- resistance pattern:-



Percentages of multiple drug resistance in E. coli isolates for each group are given in Figure. Among the isolates from male patients, 79% (15/19 isolates) exhibited resistance to two or more antimicrobials. Moreover, the resistance to four or more antibiotics occurred at a frequency of 47%. Twelve of the isolates were resistant to nine antibiotics (CXM/NA/PB/E/CIP/ AMC/CFS/NX/LE).Among the isolates from female, 70% (22/31) exhibited resistance to two or more antimicrobials. There was no significant difference in multi-drug resistance between isolates from male patients and female patients. The rates of antibiotic resistance to four or more antibiotics (51%) were similar to the rates of male patients. However, the number of resistant antibiotics was higher in the E. coli isolates from the males than the female patients. Resistance to over nine antibiotics was detected only in female patients. The highest rate of resistance was to 10 antibiotics (CXM/CX/NA/AK/LE/NX/ CAZ/PIT/CIP/E). Resistance to CXM was the most frequently observed in isolates.

4.Statistical analysis

Antimicrobial susceptibility data were expressed as percentages. A one way Analysis of variance or chi square statistics was used to estimate the overall difference between the percentages or frequencies of resistance of E. coli isolates. In all cases, $p < 0.05$ was regarded as statistically significant.

DISCUSSION

The etiological agent is mainly *E.coli* but nowadays *klebsiella, pseudomonas, proteus, Enterococcus* have been reported increasingly from UTI. In our study the rate of isolation for these pathogens was low. May the risk of infection with these organisms are more in hospital environment where noncosmial infection plays a big role. [7, 8]The % of isolates of *E.coli* was 66.6%. This is an agreement with Jha et al and Fadd et al (3, 4).*E.*coliwere found to be multidrug resistant. Resistance was seen in IIIrd generation cephalosporins. This is disturbing as this may be due to indiscriminate use of antibiotics or over the counter sales these patients were not hospitalized. So question of noncosmial infection does not arise. This was also seen by (B Das et al & al tawfiq et al., 2015). Enterococci was resistant to cephalosporin for too much extent. ESBL is an enzyme produced by gram negative bacilli like E.coli, klebsiella. They can produce large amount of extended spectrum Beta lactamase enzyme which hydrolyse oxymino beta lactamase like cefotaxime, ceftriaxone and ceftazidime. These plasmid oriented enzymes confer multidrug resistance. Now a days also found in nonhospitalized patients as seen by (smita jaiswal et al., 2016). So ESBL study may throw more information on this resistance. This can monitor the therapy for resistant bacteria.

CONCLUSION:-

Urine infection is one of the most common medical problems affecting the population. This retrospective study was performed with the aim of surveying the incidence of antibiotic resistance patterns of bacterial agents, isolated from patients with UTI-positive cultures between June 2018 and august 2018 in KPC medical college & hospital Kolkata. In the present study, 75 patients with UTI-positive cultures were identified, 50 cases (66.6%) were female and 25 cases (33.3%) were male.

E. coli was identified as the predominant cause of UTIs (66.6%), followed by *klebsiella pneumoniae* (20.0%), *Pseudomonas aeruginosa* (8%), *Enterococcus faecalis* (2.66%), *Staphylococcus aureus* (1.34%), CONS (1.34%). Similar observations have been reported in a previous study. The most effective antibiotics against *E. coli* were nitrofurantoin (80.25%), netilamicin (82.05%), Tobramycin (86.7%), cefactor (79.25%) & meropenem (68.15%). Meropenem is a carbapenem antibiotic, which is highly stable against lactamase hydrolysis. From the present study, it appears that carbapenem is the drug of choice for serious infections with *E. coli* organisms as has been recommended earlier. However, polymyxin-B is the most powerful drug against *klebsiella pneumonia*&*Pseudomonas aeruginosa.* In a recent study, it has found that klebsiella spp showed a high degree of Sensitivity to colistin, piperacillin/tazobactum and nirofurantoin. Cefuroxime and cefoxitin resistance rate of *E coli* was 78% and 67% respectively. The present results are similar to previous studies conducted in several countries such as Lebanon (88%), Iran (88%) and Taiwan (80%). A study in Kolkata showed that the lowest percentage of susceptibility was manifested against cefuroxime (between 21 and 32%) followed by norfloxacin (between 37 and 35%).Our data show an increase on the resistance of *E.coli* against cefuroxime. The reason for the high resistance to some antibiotics observed in this study may due to the improper use of antibiotics. The other is incorrect and unreasonable antibiotics prescription. Considering time, the appropriate dose and manner of administration are the most important aspects of rational drug prescription. Studies have shown that 30%–60% of the prescribing and use of antibiotics has been improper. However, the overtreatment of antibiotics may result in antibiotic resistance. Knowledge of antibiotic resistance patterns in *E. coli* is very important in selecting an empirical antimicrobial therapy. In order to reduce the incidence of UTIs, appropriate use of antibiotics is proposed.

CORRELATION WITH OTHER RELEVANT STUDIES:

In the study Urinary Tract Infections due to Resistant Enterobacteriaecae: Laboratory of enteric infections of Korean center for disease control and prevention in the year 2017 and reviewed independently by Falagas and Martin of Division of biomedical and clinical sciences L Sacco, university of Milan, Laboratory of enteric infections of Korean center for disease control and prevention Korea. Enterobacteriaecae were the major causes of UTIs (88%) and prevalence of MDRE UTIs among patients were, 19% overall.

In another study carried out in the Department of Microbiology, AK clinic, Mandavelipakkam, Chennai India.[3] A total of 2941 urine samples were received for culture and sensitivity during the study period. Among these, 547 samples (18.5%) yielded significant bacteriuria; 2323 samples (79.1%) showed no growth and 74 samples (2.4%) showed mixed growth. The isolates were sensitive to amikacin (82.6%), piperacillin-tazobactum (78.2%), nitrofurantoin (82.1%) and imipenem (98.9%); the sensitivity to ampicillin, cefuroxime, ceftriaxone, norfloxacin, ciprofloxacin varied from 11-25 per cent.

In the present study out of 100 samples 40 samples were multidrug-resistant enterobacteriaecae i.e. approximately 40% this difference in percentage in the number of cases is attributable to selection of cases, the present study is based only on samples drawn from cases Laboratory of enteric infections of Korean center for disease control and prevention, Korea and isolates obtained from urine samples among all hospital inpatients suspected to have, admitted during the period of August 2016 to July 2017 at AK clinic, Mandavelipakkam, chennai, India. But in both the studies the pattern of drug resistance has shown considerable similarity with the present study in terms of multi drug resistance. Urinary tract infections are common infections that occur worldwide and have a significant health, economic and financial burden on society. Bacteria which cause UTI, most commonly *E. coli*, are becoming more resistant to currently prescribed antimicrobials. Therefore, monitoring antimicrobial resistance patterns is an essential part of guiding therapy in patients with UTI and can also provide data which can be used for the development of policies aimed at controlling further development and spread of Antimicrobial resistance in *E. coli.*

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