STUDIES ON RESISTANCE/SENSITIVITY PATTERN OF BACTERIA RELATED WITH URINARY TRACT INFECTIONS

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SUMMARY: In order to determine the resistance pattern against five different groups of antimicrobial agents, six different species of bacteria were isolated from among two hundred cases of UTIs. The front line antibiotics for treating urinary tract infection due to Escherichia coli, Klebsiella species, Staphylococcus aureus and Proteus species should include Cephradine, Ofloxacin, Cefaclor, Cephalothin and Pipemidic acid. However, for treating UTI due to Pseudomonas species, the drug of choice must be a member of fluoroquinoline group (norfloxacin, ofloxacin and ciprofloxacin).

Key Words: Urinary infections, E. coli, Staph Aureus, Proteus.

INTRODUCTION

Urinary tract infections (UTIs) are the most common infections experienced by both male and female and are particularly responsible for discomfort in elderly patients, thus representing a risk of bacteremia, septic shock, respiratory distress syndrome and death (7). The susceptibility of the host and the presence of urinary tract pathogens are of primary importance in the development of infection. Common pathogens include *Escherichia coli*, *Proteus species*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterococci* and *Satphylococcus saprophyticus* (17).

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Treatment with antimicrobials should eradicate the infecting organisms. These agents must exhibit maximum activity against the major pathogens as well as low potential for the development of bacterial resistance. Antibiotic resistance represents a serious problem for clinicians and great effort is being made to control the resistance. A number of studies carried out in previous years indicate an increased frequency of resistance of urinary tract infecting organisms to common antibiotics (4,6,10,11,13-16). Keeping in view the importance of UTI, two hundred bacterial cultures were screened for their sensitivity/resistance patterns to different antimicrobials. The organisms were isolated from urinary tract infections of general practice patients population of Karachi region. An attempt is being made to design effective therapy for the management of resistant pathogens.

MATERIALS AND METHODS

Sensitivity medium: Muller Hinton Agar (12).

Medium for inoculation: Muller-Hinton Broth (12).

MacFarland turbiditiy standard (12)

Preparation of MacFarland turbidity standard: 0.5 ml of 0.048 M barium chloride was added to 99.5 ml of 0.36 N sulfuric acid. 5 ml of it was aliquoted into screw-capped tubes of same size which were used in the procedure. The standard was stored in dark, at room temperature and was vortexed prior to use.

Assay plates

Flat bottom petri dishes with internal diameter of 150 mm were used in the procedure. Bottles containing MHA were allowed to equilibrate to 50°C in a water bath. The medium was poured into sterile petri dishes on a level surface to a depth of 4 mm and allowed to harden. The pH of the medium was adjusted to 7.2. The medium was allowed to dry for 15-30 minutes before use or stored at 4°C.

Sterile swabs

Sterile swabs available commercially were used for the inoculation of the organisms into the petri dishes.

Preparation of inoculum

Portions of each of four or five well isolated colonies of same morphological type were touched with a sterile wire loop, suspended into tubes containing 5 ml of MHB. The medium was then incubated at 35°C for 2-8 hours until the turbidity reached or exceeded that of 0.5 MacFarland standard (already prepared). If the suspension was exceeded, it was diluted with broth it was visually comparable to the 0.5 MacFarland standard.

Table 1: Zone diameter interpretive standard of antimicrobial agents used in the study.

Antimicrobials	**Disc Content**	**Resistant**	**Intermediate**	**Susceptible**
Cephalosporin Group				
Cephalothin	30ug	<14	15-17	>18
Cefaclor	30ug	<14	15-17	>18
Cephradine	30ug	<14	15-17	>18
Cefuroxime (Na)	30ug	<14	15-22	>23
Cefixime	05ug	<15	16-18	>19
Quinolone Group				
Nalidixic acid	30ug	<13	14-18	>19
Pipemidic acid	50ug	<11	15-22	>23
Fluoroquinolone Group				
Norfloxacin	10ug	<12	13-16	>17
Ofloxacin	05ug	<12	13-15	>16
Ciprofloxacin	05ug	<15	16-20	>21
Semisynthetic Penicillin				
Ampicillin				
1. when testing Gram negative enteric organism	10ug	<13	14-16	>17
2. when testing Staphylococci	10ug	<28	--	>29
Amoxycillin	10ug	<13	14-16	>17
Combined Therapy				
Amoxycillin plus Clavulanic acid				
1. when testing Staphylococci	20/10ug	<19	--	>20
2. when testing other organisms	20/10ug	<13	14-17	>18
Co-trimoxazole: Trimethoprim plus Sulfamethaxazole	1.25/23.75ug	<10	11-15	>16

Table 2: Antibiotic susceptibility of the organisms isolated from urinary tract infections.

S.	Antimicrobial agents	Name and total percentage of organism											
		Escherichia coli (52%)		*Klebsiellla sp.* (29%)		*Pseudomonas aeruginosa* (7%)		*Staphylococcus aureus* (7%)		*Proteus sp.* (4%)		*Serratia marceaces* (1%)	
		S	R	S	R	S	R	S	R	S	R	S	R
01	Amicolox, Ampicillin / Cloxacillin	25	27	08	21	02	05	07	00	03	01	01	00
02	Norfloxacin	44	08	26	03	05	02	07	00	04	00	01	00
03	Cephradine	48	04	23	06	02	05	07	00	04	00	00	01
04	Ofloxacin	46	06	28	01	05	02	07	00	04	00	01	00
05	Cefuroxime (Na)	43	09	26	03	01	06	07	00	04	00	01	00
06	Amoxycillin/ Clavulanic acid	46	06	23	06	02	05	07	00	04	00	01	00
07	Ciprofloxacin	43	09	26	03	06	01	07	00	04	00	01	00
08	Ampicillin	21	31	06	23	02	05	07	00	03	01	01	00
09	Nalidixic acid	38	14	24	05	02	05	07	00	04	00	01	00
10	Cefaclor	44	08	23	06	02	05	07	00	04	00	00	01
11	Amoxycillin	24	28	07	22	02	05	07	00	03	01	00	01
12	Cefspan	-	-	-	-	-	-	-	-	-	-	-	-
13	Cephalothin	42	10	20	09	01	06	07	00	03	01	00	01
14	Pipemidic acid	40	12	26	03	06	01	07	00	04	00	01	00
15	Co-trimoxazole	13	39	16	13	02	05	04	03	03	01	01	00

Inoculation of MHA plates

A sterile swab was dipped into the broth suspension of the organisms within 15 minutes of standardization. Excess inoculum was removed by rotating the swab several times against the wall of the tube above the fluid level. The entire surface of an MHA plate was then streaked evenly in three/two directions approximately 60 degrees from each other.

Quinolones

In the present study pipemidic acid was found more effective than nalidixic acid. The resistance observed by pipemidic acid was 23% due to *E. coli*, 10.3% due to *Klebsiella* and 14.2% due to *Pseudomonas aeruginosa*. While the resistance of nalidixic acid was 26.9% due to *E. coli*, 17.2% due to *Klebsiella*, and 71.4% due to *Pseudomonas aeruginosa*.

Fluoroquinolone group

The member of this group behaved more or less in a similar pattern and were found highly effective against all the species isolated from UTI. However, Ofloxacin had an edge over other fluoroquinoloness against *E. coli*.

Semisynthetic penicillins

The Gram positive pathogens were found sensitive to both ampicillin and amoxycillin while the Gram negative pathogens were most found resistant. The result of the present study indicated *E. coli*, resistance to ampicillin as 59% and to amoxycillin as 53.8%. Similarly *Klebsiella* which are second major isolates exhibited even higher resistance than *E. coli* (ampicillin 79.3% and amoxycillin 75.8%). Similar results were noted with *Pseudomonas aeruginosa* (ampicillin 71.4% and amoxycillin 71.4%). However, the Gram positive isolates i.e. *Staphylococcus aureus* and *Proteus sp.* were sensitive to both ampicillin and amoxycillin. As far as Gram negative pathogens are concerned the present study indicated that 52% of *E. coli* was resistant to ampicillin.

Combined therapy

In the present study three combinations of antimicrobial were included which are frequently used by general practitioner.

Trimethoprim/sulfamethoxazole (co-trimoxazole): Except *Serratia marcescens*, all pathogens indicated moderate to high degree of resistance against this combination.

Table 3: Percentages of resistant pattern of organisms isolated from urinary tract infections.

	Escherichia coli	*Klebsiella sp.*	*Pseudomonas Aeruginosa*	*Staphylococcus aureus*	*Proteus sp.*	*Serratia marcences*
Cephalosporins						
Cephalothin	19.20	31.00	85.70	00.00	25.00	100.00
Cefaclor	15.30	20.60	71.40	00.00	00.00	100.00
Cephradine	07.60	20.60	71.40	00.00	00.00	100.00
Cefuroxime (Na)	17.30	10.30	85.70	00.00	00.00	00.00
Cefixime	30.70	24.10	85.70	85.70	00.00	00.00
Quinolones						
Nalidixic acid	26.90	17.20	71.40	00.00	00.00	00.00
Pipemidic acid	23.00	10.30	14.20	00.00	00.00	00.00
Fluoroquinolones						
Norfloxacin	15.30	10.30	28.50	00.00	00.00	00.00
Ofloxacin	11.50	03.40	28.50	00.00	00.00	00.00
Ciprofloxacin	17.30	10.30	14.20	00.00	00.00	00.00
Penicillins (semi-synthetic)						
Ampicillin	59.00	79.30	71.40	00.00	25.00	00.00
Amoxycillin	53.80	75.80	71.40	00.00	25.00	100.00
Combined Therapy						
Ampicillin/Cloxacillin	51.90	72.40	71.40	00.00	25.00	00.00
Amoxycillin/Clavulanic acid	11.50	20.60	71.40	00.00	00.00	00.00
Co-trimoxazole	75.00	44.80	71.40	42.00	25.00	00.00

Ampicillin/cloxacillin: This combination seemed to be less useful for UTIs caused by Gram negative pathogens. However, the Gram positive pathogens did show 100% sensitivity.

Amoxycillin/Clavulanic acid: The efficacy of this combination seems to be much better than the other two combinations. However, this combination also did not prove its effectiveness against *Pseudomonas aeruginosa* (71.4% resistance).

Disk placement

The antimicrobial impregnated disks were placed with sterile forceps on to the agar surface in such a way that each disk was at least 24 mm from the other disk to avoid overlapping during incubation. The plates were then incubated at 37°C for 18-24 hours.

Results and interpretation

At the end of incubation period, the diameter of zones of inhibition around each disk was measured with a vernier caliper on the back of the plate, with reflected light against a dark non-reflective background. The zone diameter for each antimicrobial agent was then interpreted as resistant/sensitive as given in Table 1.

RESULTS AND DISCUSSION

Six different Genra of bacteria, isolated in a previous study were subjected for antibiotic sensitivity determination. The percentage of various organisms isolated were *Escherichia coli* 52%, *Klebsiella sp.* 29%, *Pseudomonas aeruginosa* 07%, *Staphylococcus aureus* 07%, *Proteus* 04% and *Serratia marcences* 01%. These organisms were isolated from urinary tract infections of general practice patient populations. The antibiotic sensitivity of different organisms was carried out on diagnostic sensitivity plates (DST) by standard disk diffusion technique approved by National Committee for Clinical Laboratory Standard (12). Resistant pattern of the organisms isolated from UTIs are presented in Tables 2 and 3 respectively.

Cephalosporin group

Among Cephalosporins, Cephradine - a first generation Cephalosporin was most effective for *E. coli*. Only

7.6% of the strains screened were found resistant to Cephalosporin. The resistant percentages of other cephalosporins were recorded as follows, cefaclor 15.3%, cefuroxime 17.3%, cephalothin 19.2% and cefixime 30.7%. However, for *Klebsiella sp.*, cefuroxime was found to be more effective than the other four cephalosporins. The *Proteus sp.*, tested in the study were found sensitive to all the cephalosporins used except cephalotin (25% resistant). Among Gram negative species only *Pseudomonas aeruginosa* were found to be resistant to all the cephalosporin tested. *Staphylococcus aureus* were found sensitive to all cephalosporins except cefixime (85.7% resistant) where as the only strain of *Serratia marcences*, isolated indicated sensitivity to cefuroxime (Na) and cefixime (Table 3).

The conclusions which can be drawn from the present studies are:

i. *E. coli* is still the predominant pathogens of UTIs (52%). This is inconstant with the results of the studies carried out by Ahmed *et al.* (1) and Farooqui *et al.* (5) from Karachi region.

ii. In recent years infections due to *Klebsiella* has been significantly increased (upto 29%). In present study they are shown next to *E. coli* causing UTI.

iii. Resistance to UTIs pathogens is rapidly increasing to co-trimoxazole (Previous 60%, present 75%).

iv. Front line antibiotics for treating UTIs should include cephradine, ofloxacin, cefaclor, cephalothin, pipemidic acid. However, for treating UTI due to *Pseudomonas* the drug of choice must be a member of fluoroquinolone group (ofloxacine, ciprofloxacin, or norfloxacin).

v. As far as combined therapy is concerned their effectiveness is not superior to single antimicrobial agent.

Finally, the study points to the need of frequent monitoring of bacterial spectrum and their sensitivity pattern in UTI so that the indiscriminate use of antibiotic therapy could be checked.

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