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Antibiograms Of Bacterial Flora Isolated From Patients With Urinary Tract Infections−A Study In Garhwal Region

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Abstract

Introduction: Bacterial presence in urinary tract infections (UTIs) is frequently reported. Several bacterial pathogens especially the members of Entero bacteriacae, Staphylococcus spp., Enterococci, other coliforms and Pseudomonas aeruginosaare commonly associated with urinary tract infections. Assessment of antibiotic resistance in prevailing bacterial flora is a crucial step in context of thein tricacies of developing resistance.

Aim of the study: The present study was conducted to isolate and identify the bacterial flora along with antibiogram profiling of the pathogenic isolates against routine antibiotics from patients with urinary tract infection sat HNB Base Hospital, Srinagar, Garhwal.

Materials & Methodology: A total of 816urine samples from clinically suspected patients (including 346 males and 470 females) of UTI were studied. Sample collection, Isolation and biochemical identification of aerobic bacteria followed by antibiotic sensitivity profiling of the pathogenic isolates were done using standard protocols.

Result & Conclusion of the study: Out of 816 samples collected, 337 bacterial pathogens were isolated. Among Gram-negative pathogens (48.96% of total isolates), Escherichia colialone accounted for 21.06% of the isolates followed by Klebsiellaspp. (10.38%),

Acinetobacter spp.(5.34%), Enterobacter spp, Pseudomonas spp.,Proteus spp.(2.67% each), Citrobacterspp (2.07%), Salmonella spp.(1.1%) and Morganella spp.(0.8%). Among Gram-positive cocci (51.03%of total isolates), Enterococcus (25.51%) were more frequently isolated than Coagulase-negative Staphylococcus (CONS) (18.39%), Streptococcus (4.15%) and Staphylococcus aureus(2.96%). Morganella spp. And Enterobacter spp. were the most resistant pathogens among Gram-negative bacterial isolates.Among Gram-positive bacterial isolates, CONS along with Streptococci were the most resistant pathogens.

Keywords: Bacterial flora. Urinary tract infections. Antibiotic sensitivity profile. Coagulase negative Staphylococcus (CONS), Enterococci spp. Patients of Garhwal region.

Introduction:

Urinary tract infections (UTIs) are among the most common symptoms which bring majority of patients to hospital. UTI is an inflammatory disorder of urinary tract caused by abnormal growth of pathogens (Prakash et al, 2013). It is further divided into upper tract infections involving kidney (pylonephritis) & lower tract infections involving bladder(Cystitis), urethra (urethritis) and prostate (prostatitis). Bacterial urinary tract infections commonly cause pyelonephritis and Cystitis. Members of Enterobacteriacae are generally associated with urinary tract infections. Bacterial presence in urine is predominated by Escherichia coli, Klebsiellaspp, Proteus mirabilis, Staphylococcus saprophyticus, Staphylococcus epidermidis, Enterococci, other coliforms and Pseudomonas aeruginosa (Greenwood et al, 2012).In concordance to the global scenario, the occurrence of bacterial urinary tract infections in India is generally more common in females as compared to males (Prakash et al, 2013).

Since a long time after the introduction of antibiotics the bacteria have developed drug resistance to most recent and effective antibiotics. Increasing drug resistance has not only threatened life with resulting treatment failures but also has brought society on the verge of serious concerns regarding the better treatment strategies. Various types of drug resistance are implicated and several of them have been studied in considerable depth. Multidrug resistance (MDR), which is a major global threat for public health, arises mostly from inappropriate antibiotic use and substandard drug usage. MDR is defined as non-susceptibility to at least one agent in three or more antimicrobial categories while XDR is defined as extensively drug resistant or non-susceptibility to at least one agent in all but two or fewer categories (i.e. bacterial isolates remain susceptible to only one or two categories) (Kaur et al, 2015 & Magiorakos et al, 2012).Bacteria of Enterobacteriacae family and other bacteria like Pseudomonas produce Class C β-lactamases and Amp C beta-lactamases (Amp C) are one of their classes. These Amp C beta lactamase have capability of hydrolyzing Cephalosporins without being inhibited by beta-lactamase inhibitors like clavulanic acid, sulbactam and Tazobactam (Bush et al, 2010) thus they are resistant to narrow, broad spectrum cephalosporin to Cephamycin like Cefoxitin, Aztreonam and beta-lactamase inhibitors (Thomson, 2010). Macrolide, Lincosamide, Streptogramin (MLS antibiotics) though chemically distinct but have similar effect on bacterial protein synthesis in gram positive isolates. Macrolide-lincosamide-streptogramin B resistance (MLSB resistance) is another example which is the resistance

developed mainly in Methicillin-resistant Staphylococcus aureus (MRSA) against Clindamycin & lesser in CONS & Enterococci (Lim et al). Strains with inducible resistance to clindamycin appear in vitro clindamycin sensitive until tested with erythromycin disc proximation test (D zone inhibition test) as described in CLSI guidelines (CLSI guidelines, 2017). While strains with constitutive MLSB resistance appear resistant to both Clindamycin & Erythromycin (Marjini et al, 2015).

Materials & Methods:

Collection of specimens:
A total of 816urine specimens from clinically suspected patients (including 346 males and470 females) of UTI were taken at HNB Base Hospital, Srinagar, Garhwal for over a period of one year (September 2017 to August2018).The samples were collected in sterile containers using standard protocols.

Sampling procedure: Early morning midstream urine samples were advised to be collected with aseptic precautions. Samples collected were mostly inoculated immediately and very few of them were kept in refrigerator for 2-3 hrs until inoculation.

Isolation and identification of bacterial isolates:
Aerobic bacterial isolates were identified and isolated using morphological examination and microscopic identification followed by biochemical and aerobic culture methods. The samples were first inoculated on Blood agar and MacConkey agar plates with the help of inoculation loop. After inoculation, the blood agar plates were subjected to incubation at 37°C for 24 hours. After incubation, all unique colonies were sub-cultured to get isolated colonies and sufficient inoculums were taken for preservation of colonies in BHI agar slants. Blood agar slant were also used for preserving fastidious colonies. Gram's staining followed by microscopic examination was performed for differentiation of Gram-positive and Gram-negative bacteria. Biochemical identification of the isolates was done using biochemical tests recommended for identification of aerobic bacteria as per standard methods (as described by Prescott et al, 2003, Koneman et al, 1997).

Antibiotic sensitivity profiling of the pathogenic isolates:
Pathogenic isolates were subjected to the antibiotic sensitivity testing against routine antibiotics (results; Table-1& 2)using Kirby Bauer disc diffusion method as recommended by the Clinical and Laboratory Standard Institute (CLSI). Antibiotic discs were procured from Hi Media. Inoculum density was kept as approximately $1x10^8$ CFU/ml. The inocula were adjusted to McFarland 0.5 turbidity standards. Mueller Hinton Agar (MHA) was used throughout the testing. The inocula were spreaded on the agar in Petri plates with the help of sterile cotton swab sticks, antibiotic discs were placed on to the surface. Plates were incubated at 37 °C for 24 hours. The inhibition zones were measured in mm.

MRSA isolates were detected by using Cefoxitin (30 µg discs) according to CLSI guidelines, Anand et al, 2009, and Furtado et al, 2014. As also recommended and described by

Felten et al, 2002 that Cefoxitin or Moxalactam are best screening antibiotics for routine detection of MRSA, being preferable to oxacillin screening agar test (Cauwelier et al, 2004), Cefoxitin disc with inhibition zone of < or equal to19 mm was considered as Methicillin resistant while inhibition zone of> or equal to 20 mm zone diameter was considered as Methicillin sensitive.

For Amp C beta-lactamases producers we used Cefoxitin 30 µgm as screening antibiotic less than and equal to 18 mm (Gupta et al, 2014) and Cefoxitin+ Cloxacillin(100µgm) double disc synergy method (DDS method) where greater than 5 mm or equal zone of inhibition was considered to be positive (Brenwald et al, 2005).

D zone test was performed for detecting Macrolide –Lincosamide – Streptogramin B resistance (MLSB). The Erythromycin & Clindamycin Double Disc susceptibility test was performed for Staphylococcus including CoNS and Enterococci (CLSI guidelines, Prabhu et al, 2011&Fiebelkorn et al, 2003)

Result and discussion:

Prevalence of bacteria in the samples:

Out of 816 samples collected, 337 bacterial pathogens were isolated. Among Gram-negative pathogens (48.96% of total isolates), Escherichia colialone accounted for 21.06% of the isolates followed by Klebsiellaspp.(10.38%), Acinetobacter spp.(5.34%), Enterobacter spp, Pseudomonas spp., Proteus spp.(2.67% each), Citrobacterspp (2.07%), Salmonella spp.(1.1%) and Morganella spp.(0.8%).Among Gram-positive cocci (51.03%of total isolates),Enterococcus (25.51%) were more frequently isolated than Coagulase-negative Staphylococcus (CONS) (18.39%), Streptococcus (4.15%) and Staphylococcus aureus(2.96%). Overall prevalence of the bacterial isolates in urine samples along with their MDR/XDR status and type of resistance observed is shown in **Table-1.**

Table-1: Overall prevalence of the bacterial isolates in urine samples

S. no.	Pathogen	Number of isolates	Prevalence (%)	MDR/XDR	Type of MDR resistance
1	Enterococcus spp.	86	25.51%	MDR: 82.5% XDR:2.3%	D test+ve:2 MRE*:48 VRE**: 6
2	Escherichia coli	71	21.06%	MDR:6.05% XDR:7.04%	Amp C resistance: 2
3	CONS	62	18.39%	MDR:4.83% XDR:1.6%	D test+ve: 1 MRSE#:22 VRSE##:1 Amp C: 1
4	Klebsiellaspp.	35	10.38%	MDR: 2.85% XDR:28.57%	Amp C resistance: 1

5	Acinetobacterspp.	18	5.34 %	MDR:72.2% XDR:5.5%	Amp C resistance: 2
6	Streptococcus spp.	14	4.15%	MDR:42.8% XDR:14.28%	D test+ve: 1 all vancomycin sensitive MRS▪: 5
7	Staphylococcus aureus	10	2.96%	MDR:80% XDR:0%	D test+ve: 1 MRSA▪▪: 3Amp C: 2all vancomycin sensitive
8	Enterobacter spp.	9	2.67%	MDR:8.89% XDR:0%	Amp C resistance: 2
9	Pseudomonas spp.	9	2.67 %	MDR:77.7% XDR:11.11%	-
10	Proteus spp.	9	2.67 %	MDR:6.66% XDR:11.11%	Amp C producers: 1
11	Citrobacterspp.	7	2.07 %	MDR:1.42% XDR:14.2%	Amp C producers: 1
12	Salmonella spp.	4	1.1%	MDR:50% XDR:0%	-
13	Morganellaspp.	3	0.8 %	MDR :33% XDR:0%	-

MRE*:Methicillin resistant Enterococci, VRE**: Vancomycin resistant Enterococci, MRSE#:Methicillin resistant Enterococci, VRSE##:Vancomycin resistant Enterococci, MRS▪: Methicillin resistantStreptococcus spp., MRSA▪▪: Methicillin resistant Staphylococcus aureus.

A comparison of prevalence of the bacterial isolates in present study with few other similar studies is presented in Table-2

Table-2: A comparison of prevalence of the bacterial isolates in present study with few other similar studies.

S. No.	Study	No. of specimens/ patients studied	No. of bacteria isolated	Prevalence of bacteria
1	Present Study	816	337	Among Gram-negative pathogens (48.96% of total isolates), E. coliwere 21.06% of all the isolates followed by Klebsiellaspp.(10.38%), Acinetobacter

				spp.(5.34%), Enterobacter spp, Pseudomonas spp., Proteus spp.(2.67% each), Citrobacterspp (2.07%), Salmonella spp.(1.1%) and Morganella spp.(0.8%). Among Gram-positive cocci (51.03%of total isolates),Enterococcus (25.51%) were more frequently isolated than CONS (18.39%), Streptococcus (4.15%) and S. aureus(2.96%).
2	Jermakow et al, 2016	276	206	E.coli(52%)>Enterococci & Streptococci (25%)>Gram-negative rods other than E.coli were 21% (that included Klebsiella, Proteus spp., Pseudomonas spp., Enterobacter spp., and Serratia spp.)> CONS less than 2%.
3	Dash et al, 2013	1670	577	E.coli(68.8%) >Enterococci (9.7%) >CONS (6.2%) >S. aureus (4.9%).
4	Khawcharoenporn et al, 2013	676	492	E.coli(72%)>Klebsiella (15%) > Proteus (7%)>Enterococci (4.8%)>Pseudomonas (3.6%).
5	Pankaj Baral et al, 2012	710	219	E.coli(81.3) >Citrobacter(5%) > CONS(2.7%),Klebsiella(2.7%)>Enterobacter (1.8%).
6	Alemu et al, 2012	385	40	E.coli (47.5%)> CONS (22.5%)>S. aureus (10%)>Klebsiella (10%)>Enterobacter (5%).
7	Poulsenet al, 2012	276	49	E. faecalis(55%) >E.coli(12.12%) >Streptococcus gallolyticus (8.2%).

Antibiotic sensitivity profiles of the bacterial isolates:

Antibiotic sensitivity profiles of Gram-negative bacterial isolates are detailed in **Table-3.** Overall, among Gram-negative bacterial isolates, Morganella spp. And Enterobacter spp. were the most resistant pathogens with overall sensitivity of 34% and 43% respectively followed by Acinetobacter spp.,Citrobacter spp.,Pseudomonas spp.,Salmonella spp.,E. coli, Proteus spp., and Klebsiella spp. with overall sensitivity of 44%, 44%, 45%, 47%, 48.37%, 49%, and 49.74%respectively.

Meropenem, Amikacin, Piperacillin-Tazobactam, Cefoperazone-sulbactam and Colistin were the most effective antibiotics against Gram-negative bacterial isolates with overall efficacy of 92.1%, 80.3%, 75.8%, 75.6% and 75% respectively. Next to these were Gentamicin, Tigecycline, Cefoxitin-Cloxacillin, Cefipime, Cefotaxime, Aztreonam, Levofloxacin,Ciprofloxacin, Cotrimoxazole, Nitrofurantoin,Polymyxin-Band Cefixime with overall efficacy of64.61%, 58%, 52.44%, 48.2%, 46%, 41.2%, 38.9%, 34.9%, 32.8%, 31.9%,

30.8%and 30.5% respectively. A lesser level of antibacterial effect was shown by Cefoxitin, Ticarcillin-Clavulanic acid, Cefuroxime, Ampicillin and Amoxyclav with 22.8%, 21.3%, 15.2%, 9.8% and 8.2%overall efficacy respectively.

Table-3: Overall antibiotic sensitivity (%) in Gram-negative bacterial isolates

S. No.	Antibiotics	Bacterial Isolates								
		E. coli	Klebsiella spp.	Enterobacter spp.	Acinetobacter spp.	Proteus spp.	Pseudomonas spp.	Citrobactersp p.	Salmonella spp.	Morganellasp p.
1.	Ampicillin (10 µg)	7.1%	6.6%	0%	25%	50%	0%	0%	0%	0%
2.	Amoxyclav (20/10µg)	5.8%	0%	0%	7.14%	11.1%	0%	0%	50%	0%
3.	Amikacin (30µg)	85.7%	74.2%	100%	64.7%	85.7 %	62.5%	83.3%	100%	66.6%
4.	Gentamicin (10µg)	67.7%	74.07%	44.4%	42.8%	77.7%	75%	66.6%	66.6%	66.6%
5.	Nitrofurantoin(300µg)	76.2%	44.4%	100%	0%	0%	0%	66.6%	0%	0%
6.	Ciprofloxacin(5µg)	24.2%	33.3%	0%	36.3%	57.1%	80%	33.3%	0%	50%
7.	Levofloxacin (5µg)	31.03%	45.4%	0%	57.1%	40%	60%	16.6%	50%	50%
8.	Cotrimoxazole (1.25/23.75µg)	25.5%	54.2%	20%	27.7%	40%	28.5%	16.6%	33.3%	50%
9.	Cefuroxime (30µg)	13.5%	46.6%	20%	0%	42.8%	0%	14.2%	0%	0%
10.	Cefoxitin (30µg)	62.8%	33.3%	40%	0%	55.5%	0%	14.2%	0%	0%
11.	Cefoxitin - cloxacillin (100µg)	68.5%	38.8%	80%	28.5%	77.7%	0%	28.5%	100%	50%
12.	Cefotaxime (30µg)	25.5%	51.8%	50%	47.05%	85.7%	20%	33.3%	50%	50%
13.	Cefixime (30µg)	19.1%	45%	16.6%	50%	77.7%	16.6%	0%	50%	0%
14.	Cefeparazone - sulbactam (75/10µg)	83.3%	65.5%	71.4%	87.5%	80%	87.5%	80%	75%	50%
15.	Cefipime (30µg)	34.7%	52.4%	16.6%	50%	50%	71.4%	75%	33.3%	50%

16.	Piperacillin-Tazobactam (100/10µg)	66.6%	69.2%	71.4%	76.9%	88.8%	87.5%	80%	75%	66.6%
17.	Ticarcillin-Clavulanic acid (75/10µg)	17.8%	33.3%	0%	12.5%	28.5%	75%	0%	25%	0%
18.	Meropenem (10µg)	92.4%	76.9%	88.9%	93.7%	87.5 %	90%	100%	100%	100%
19.	Tigecycline(15 µg)	86%	88.4%	100%	86.6%	0%	62.5%	33.3%	66.6%	0%
20.	Aztreonam (30µg)	26.2%	44.4%	0%	33 %	40%	57.1%	20%	50%	100%
21.	Colistin (10µg)	100%	100%	100%	100%	0%	75%	100%	100%	0%
22.	Polymyxin-B (300U)	44.4%	16.6%	33.3%	50%	0%	33.3%	100%	0%	0%

Antibiotic sensitivity profiles of Gram-positive bacterial isolates are detailed in **Table-4.** Overall, among Gram-positive bacterial isolates, CONS along with Streptococci were the most resistant pathogens with overall sensitivity of 11.1% and 20% respectively followed byS. Aureus and Enterococci with overall sensitivity of 33.3%, and 45.1%respectively.

Vancomycin, Linezolid and Nitrofurantoin were the most effective antibiotics against Gram-positive bacterial isolates with overall efficacy of 96% each followed by Meropenem and Tigecycline with overall efficacy of 94% and 86.4% respectively. Cefoperazone-sulbactam, Chloramphenicol, Cefoxitin-Cloxacillin, Gentamicin, Amikacin, Cefipime, Cefotaxime and Levofloxacin showed an overall efficacy of 73%, 72.7%, 61%, 59%, 54%, 51%, 46.8% and 42.6%,respectively, while Clindamycin,Cotrimoxazole, Cefoxitin, Cefixime, Amoxyclav, Teicoplanin, Ampicillin and Cephalexin exhibited the overall efficacy of 37%, 32%, 31%, 30%, 27.8%, 27.4%, 26% and 22%respectively.Azithromycin, Ciprofloxacin and Erythromycin were the least effective with overall efficacy of 17%, 16% and 12% respectively.

Table-4: Overall antibiotic sensitivity (%) in Gram-positive bacterial isolates

S. No.	Antibiotics	Bacterial Isolates			
		S. aureus	Coagulase negative staphylococci (CONS)	Enterococci	Streptococci
1.	Ampicillin (10 µg)	0%	4.4%	32.7%	66.6%
2.	Amoxyclav (20/10µg)	12.5%	5.7%	43.07%	50%

3.	Amikacin (30µg)	60%	87.9%	19.5%	50%
4.	Gentamicin (10µg)	50%	75%	22.7%	90%
5.	Nitrofurantoin (300µg)	100%	93.3%	90%	100%
6.	Ciprofloxacin (5µg)	0%	24.3%	14.5%	25%
7.	Levofloxacin (5µg)	33.3%	54.1%	16.6%	66.6%
8.	Cotrimoxazole (1.25/23.75µg)	50%	26.08%	24.3%	27.3%
9.	Chloramphenicol (30µg)	71.4%	77.2%	53.6%	88.8%
10.	Cefoxitin (30µg)	25%	25%	18.5%	54.5%
11.	Cefoxitin-Cloxacillin(100µg)	75%	71.8%	24.07%	72.7%
12.	Cefotaxim (30µg)	33.3%	30.7%	23.5%	100%
13.	Cephalexin (30µg)	0%	25%	14.3%	50%
14.	Cefeparazone-Sulbactam (75/10µg)	75%	83.3%	33%	100%
15.	Cefixime (30µg)	0%	5.8%	15%	100%
16.	Cefipime (30µg)	50%	28.5%	25%	100%
17.	Clindamycin (2µg)	40%	64.9%	10.7%	33.3%
18.	Erythromycin (15µg)	0%	3.3%	3.3%	40%
19.	Azithromycin (15µg)	0%	17.07%	14%	37.5%
20.	Meropenem (10µg)	75%	100%	100%	100%
21.	Tigecycline(15 µg)	66.6%	91.1%	87.9%	100%
22.	Linezolid (30µg)	100%	96.3%	97.3%	90%

23.	Vancomycin (30µg)	100%	97.1%	85.7%	100%
24.	Teicoplanin (30µg)	33.3%	11.1%	45.1%	20%

A comparison of magnitude of resistance observed in present study with other similar studies is presented in **Table-5**.

Table-5: A comparison of magnitude of resistance observed in present study with other similar studies.

S. No.	Study	No. of specimens / patients studied	No. of bacteria isolated	Antibiotic resistance patterns
1	Present Study	816	337	**Overall sensitivity; Gram-negative bacterial isolates:** Morganella spp. (34%)<Enterobacter spp.(43%) <Acinetobacter spp. (44%) <Citrobacter(44%) spp. <Pseudomonas spp. (45%) <Salmonella spp. (47%) <E. coli(48.37%) <Proteus spp. (49%)<Klebsiella spp. (49.74%). **Antibiotic efficacy against Gram-negative bacterial isolates:** Amoxyclav(8.2%) <Ampicillin (9.8%)<Cefuroxime (15.2%) <Ticarcillin-Clavulanic acid(21.3%)<Cefoxitin (22.8%)<Cefixime (30.5%)<Polymyxin-B (30.8%) < Nitrofurantoin(31.9%) <Cotrimoxazole, (32.8%) < Ciprofloxacin (34.9%) <Levofloxacin(38.9%) <Aztreonam(41.2%) <Cefotaxime (46%) <Cefipime (48.2%) <Cefoxitin-Cloxacillin (52.44%) <Tigecycline (58%) < Gentamicin (64.61%)<Colistin (75%) <Cefoperazone-sulbactam (75.6%) <Piperacillin-Tazobactam(75.8%) < Amikacin (80.3%)<Meropenem (92.1%). **Overall sensitivity; Gram-positive bacterial isolates:** CONS (11.1%) <Streptococci(20%)<S. aureus (33.3%) <Enterococci(45.1%). **Antibiotic efficacy against Gram-positive bacterial isolates:**Erythromycin(12%) <Ciprofloxacin (16%) <Azithromycin (17%) <Cephalexin(22%) <Ampicillin (26%) <Teicoplanin(27.4%) <Amoxyclav (27.8%)

				<Cefixime (30%) <Cefoxitin (31%) <Cotrimoxazole (32%) <Clindamycin (37%) <Levofloxacin(42.6%) <Cefotaxime (46.8%) <Cefipime (51%) <Amikacin (54%) <Gentamicin (59%) <Cefoxitin-Cloxacillin (61%) <Chloramphenicol(72.7%) <Cefoperazone-sulbactam (73%) <Tigecycline (86.4%) <Meropenem (94%) <Vancomycin, Linezolid and Nitrofurantoin (96% each).
2	Dash et al, 2013	1670	577	Resistance (%) for E.coli: Amp(94%)>cefaclor(66.7%)>Amoxyclav (63.7%)>Cefpodoxime(58.2%) >Ciprofloxacin(53.4%)>Cotrimoxazole(51.9%). Resistance (%) for Gram-negative bacteria:Ampicillin(92.9%)>Cefaclor(63.8%)>Amoxyclav(60.7%)>Cefpodoxime(56.1%)>Cotrimoxazole(53.4%)>Ciprofloxacin(51.2%). Resistance (%) for Gram-positive bacteria: Amp(65%)>Cefpodoxime(39.2%)Cotrimoxazole(38.3%)>Cefaclor(35.8%)>Amoxyclav (19.2%)> Cipro(13.3%).
3	Khawcharoenporn et al, 2013	676	492	Among multi drug resistant Enterobactericeae: Ampicilin 99%, Levofloxacin: 72% resistant, Cotrimoxazole 77% resistant, Amoxycillin 35% resistant. Overall resistance rate for TMP-SMX, levofloxacin and nitrofurantoin was 24%, 17% and 14%, respectively including both MDR & non MDR isolates.
4	Pankaj Baral et al, 2012	710	219	E. coli: Ceftazidime& Ceftriaxone (100%)>Gentamicin (72.9%)>Amoxycillin(55.6%)>Cotrimox(54.4%)> Norflox(36.5%). Klebsiella: Amoxycillin, Ceftazidime, Ceftriaxone Chloremphenicol & Gentamicin all 100% resistant. Acinetobacter: Highest resistance was noted to Nitrofurantoin 100%. CoNS: most resistant to cephalexin, Cloxacilli, and Cotrimoxazole (33.4% each).
5	Alemu et al, 2012	385	40	All Gram-negative isolates were 100%resistant to Ampicillin & amoxicillin. CONS: Resistance for Ampicillin 88.89%, Cotrimoxazole 77.9%, Amoxyclav& Chloramphenicol 66.7% each.

6	Eshwarappae t al, 2011	5564	510 selected	Study on E.coli, Pseudomonas, Klebsiella, Enterobacter, Enterococcus, Proteus, morganella. Resistance pattern of various uropathogens to antibiotics were: Ciprofloxacillin, Norfloxacin & Ofloxacin 74.2%>Gentamicin 49.2%>Cotrimoxazole 33.5%> nitrofurantoin 28.6%>Amikacin 28%>Imipenem&Meropenem3.9%

Conclusion of the study

This study brings forth a recent trend of antibiotic resistance in bacterial pathogens commonly associated with urinary tract infections in patients of Garhwal region. The antibiotics found effective in present study warrant their use alone and in combinations to provide effective treatment for patients with urinary tract infections. The findings of this study, with concordance of several other similar studies discussed in this article sketch out the intrinsic and emerging patterns of resistance among bacterial uropathogens. There should be continuous monitoring of resistant drugs so to avoid their misuse and strict development of Antibiotic stewardship to combat antibiotic resistance.

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REFERENCES

1. Alemu, A., Moges, F., Shiferaw, Y. (2012) Bacterial profile and drug susceptibility pattern of urinary tract infection in pregnant women at University of Gondar Teaching Hospital, Northwest Ethiopia. BMC Research Notes. 5: p197
2. Anand, K. B., Agarwal, P., Kumar, S. (2009). Comparision of Cefoxitin disc diffusion test, Oxacillin screen agar, and PCR for mec A gene for detection of MRSA. Indian Journal of Medical Microbiology, 27 (1); p.27-95.
3. Baral, P., Neupane,S., Marasini, B.P. (2012). High prevalence of multidrug resistance in bacterial uropathogens from Kathmandu, Nepal. BMC Research Notes; 5: p38
4. Brenwald, N. P., Jevons, G. , Andrews, J., Ang, L.(2005) . Disc methods for detecting AmpC b-lactamase-producing clinical isolates of Escherichia coli and Klebsiella pneumoniae; Journal of Antimicrobial Chemotherapy, August.56(3); p600-1.
5. Bush, K., Jacoby G.A. (2010) Updated functional classification of β - Lactamases. Antimicrobial Agents and chemotherapy March, Vol 54. Nos 7; p969-976.
6. Cauwelier, B., Gordts, B, Deschee maecker, P. (2004). Evaluation of a disk diffusion method with cefoxitin (30 mg) for detection of methicillin resistant Staphylococcus aureus. Eur J Clin Microbiol Infect Dis May; 23(5): p389–92.

7. D., Prakash and Saxena, R. S..(2013). Distribution and antimicrobial susceptibility pattern of bacterial pathogens causing urinary tract infection in Urban Community of Meerut City, India. ISRN Microbiology, vol. 2013, Article ID 749629; 13 pages.
8. Dash M., Padhi, S., Mohanty, I. (2013). Antimicrobial resistance in pathogens causing urinary tract infections in a rural community of Odisha, India. J.Family Community Med . Jan;20 (1): p20-26.
9. Eshwarappa, M., Dosegowda, R., Aprameya, V. (2011). Clinico-microbiological profile of urinary tract infection in South India. Indian J.Nephrol. Jan-March;21(1): p30-36
10. Felten, A,,Grandry, B., Lagrange PH. (2002) Evaluation of three techniques for detection of low-level methicillin-resistant Staphylococcus aureus (MRSA): a disc diffusion method with cefoxitin and moxalactam, the Vitek 2 system, and the MRSA-screen latex agglutination test. J ClinMicrobiol, 40; p2766–71.
11. Fiebelkorn, K.R., Crawford, S.A., Mc Elmeel, M.L. (2011) Practical disc diffusion method for detection of inducible clindamycin resistance in Staphylococcus aureus, and coagulase negative Staphylococci. Journal of Clinical Microbiology Oct 2003. Vol 41(10), p 4740-4744.
12. Furtado, S., Bhat, R. M., Rekha, B., Sukumar, D., Kamath, G. H. (2014). The Clinical Spectrum & Antibiotic sensitivity Patterns of Staphylococcal Pyodermas in the community and Hospital. Indian J Dermatol. March-April; 59 (2); p.143-150.
13. Greenwood, D., Barer M., SlackR., Irwing W. Medical microbiology. (2012) A guide to microbial infections 18th edition Churchill Livingstone Elsevier.
14. Gupta, G, Tak, V., Mathur, P. Detection of AmpC β Lactamases in Gram-Negative Bacteria. J Lab Physicians 2014. 6(1); p 1-6
15. Jermakow, K., Pajaczkowska, M., Krzyzanowska, B. (2016). The growing importance of Enterococcus & Streptococcus agalactiae in uncomplicated urinary tract infections. Family Medicine & Primary Care Review. 18, 3; p250–252.
16. Kaur, D.C., Chate, S.C. Study of Antibiotic Resistance Pattern in Methicillin Resistant Staphylococcus Aureus with Special Reference to Newer Antibiotic; Microbiology Reports-Journal of Global Infectious Diseases .Apr-Jun 2015 ;Vol-7 (2): 78-84.
17. Khawcharoenporn, T., Vasoo, S., Singh, K.(2013). Urinary Tract Infections due to Multidrug-Resistant Enterobacteriaceae. Prevalence and Risk Factors in a Chicago Emergency Department. Hindwai Publishing Corporation, Emergency Medicine International; Article ID 258517, 7 pages https://www.hindawi.com/journals/emi/2013/258517/.
18. Koneman E,W., Allen SD, Janda WM, Schreckberger PC, Winn WC editors (1997). The Enterobacteriaceae. In: Color atlas & text book of diagnostic microbiology 6th Edition Lippincott Williams &Wilkins p. 211-302, P 82-86.
19. Lim, J.A., Kwon, A.R., Kim, S.K. (2002). Prevalence of resistance to macrolide, lincosamide & Streptogramin antibiotics in Gram positive cocci isolated in a Korean Hospital. Journal of Antimicrobial Chemotherapy. 49, p 489-495.
20. Magiorakos, A.P, Srinivasan A, Carey RB, Falgas M.E. (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal

for interim standard definitions for acquired resistance. Clin Microbiol Infect 18(3):pg 268–281

21. Marjani, M.F.AL.and Abdulrajaq,RA.,. (2015) Macrolide Lincosamide Streptogramin B resistance in Enterococcus spp. Isolates in Bhagdad. World Journal of Pharmaceutical Research. Vol410); p 369-376.
22. Poulsen, L.L., Bisgaard, M., Son N.T. (2012). Enterococcus and Streptococcusspp. associated with chronic and self-medicated urinary tract infections in Vietnam. BMC Infectious Diseases, p12:320.
23. Prabhu, K., Rao, S., Rao, V. (2011). Inducible Clindamycin resistance in Staphylococcus aureus isolated from clinical samples. J Lab Physicians. Jan-Jun; 3(1): p25–27.
24. Prescott, L. M., Harley, J. P., Klein, D. A. (2003). Microbiology, Fifth ed. McGraw-Hill.
25. Rice, L.B. (2008) Federal funding for the study of antimicrobial resistance in nosocomial pathogens: No ESKAPE. J. Infect. Dis. (197); p.1079–1081
26. Thomson, K.S. (2010) Extended-Spectrum-β -Lactamase, AmpC, and Carbapenemase Issues. Journal Of Clinical Microbiology, Apr., Vol. 48(4): p. 1019–1025
27. Wayne, PA,. The Clinical and Laboratory Standard Institute. Performance Standards for Antimicrobial Susceptibility Testing. 28th Informational Supplement. (2017/2018) CLSI document M100-S27/S28.: Clinical and Laboratory Standard Institute.
28. Wayne, Pa. The Clinical and Laboratory Standards Institute / NCCLS Performance standards for Antimicrobial disc diffusion tests; Approved standards. 9th ed. (2006). CLSI Document M2-M9. Wayne, Pa,. Clinical and Laboratory Standards Institute.
29. Wayne, Pa. The Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: 12th informational supplement. (2004). NCCLS document. M100-S14. NCCLS,
30. Wayne, Pa. The Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility testing, twenty first informational supplement (2011), M100-S21.Clinical and Laboratory Standards Institute.