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Antibiogram of Gram-negative bacilli (GNB) Isolated from Urinary Tract Infection (UTI)- with a Special Reference to Detection of Amp C β-lactamase Producing Gram-negative bacilli Causing UTI

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ABSTRACT

Introduction: Urinary tract infection (UTI) remains one of the most common causes of morbidity in the general population. Wide variety of organisms are implicated in its aetiology, gram-negative bacilli (GNB)being most common. UTI is treated with a variety of broad-spectrum antibiotics. Resistance to antibiotics is increasing nowadays. Gram-negative bacilli confer resistance to β-lactam antibiotics by producing enzymes like Extended-spectrum β – lactamase and Amp C β – lactamase. The present study was carried out with the aim and objectives to isolate and identify GNB causing UTI and to prepare antibiogram of these isolates. The study also includes to find out the prevalence of Amp C β – lactamase producing GNB causing UTI.

Materials and Methods: The urine samples obtained from patients having suspected urinary tract infection were processed to isolate and identify the causative organism. Antibiotic susceptibility testing was done by Kirby Bauer Disk Diffusion method for gram-negative urinary isolates in patients with significant bacteriuria. Clinical Laboratory Standards Institute (CLSI) guidelines were followed during the interpretation of results. GNB showing resistance to 3rd generation cephalosporins and resistance to cefoxitin were subjected to Amp C Disk Test to detect Amp C β – lactamase producing GNB. Minimum Inhibitory Concentration (MIC) for ceftazidime was tested for these isolates.

Results: Total of 288 samples from patients with suspected UTI were processed.214 samples were culture positive. Out of which 167 samples showed significant bacteriuria (78.03%). Gram-negative bacilli isolated were 74.85%. All GNB showed reduced susceptibility to 3rd generation cephalosporins. They were susceptible to amikacin, nitrofurantoin, imipenem, meropenem. *Pseudomonas* isolates showed resistance to imipenem and meropenem. Prevalence of Amp C β – lactamase producing GNB was 21.6%.

Conclusion: Gram-negative bacilli producing UTI were 74.85%. These isolates showed resistance to 3rd generation cephalosporins. They were susceptible to amikacin, nitrofurantoin, imipenem, meropenem. *Pseudomonas* isolates were resistant to imipenem and meropenem. Prevalence of Amp C β – lactamase producing GNB was 21.6%.

Key Words: Urinary tract infection (UTI), Gram-negative bacilli (GNB), Antibiotic Susceptibility Testing (AST), Drug resistance, Amp C β-lactamase, Amp C Disk Test

INTRODUCTION

Urinary tract infection may be caused by the invasion of bacteria and their multiplication in urinary tract[1]. Kass (1956) gave a criterion of active bacterial infection of the urinary tract according to which a count exceeding 10^5organisms / ml denotes significant bacteriuria and indicates active UTI. Contamination accounts for 10^4organisms/mland also counts less than 10^3organisms/ml[1].UTI covers a broad range of clinical entities that differ in clinical presentation, degree of tissue invasion, antibiotic requirement and epidemiological settings[2]. Patients with UTI may present with a wide range from asymptomatic infection to pyelonephritis[2]. The common symptoms of UTI are urgency and frequency of micturition along with that patient may have pain and lower abdominal discomfort. The commonest condition is cystitis, due to infection of bladder with the uropathogenic bacterium, of which *E.coli*is the most common[3]. Other organisms are *Staphylococci saprophyticus* or in hospital-acquired infections *Klebsiella spp., Proteus mirabilis*, other coliforms, *Pseudomonas aeruginosa*, or *Enterococcus faecalis*[3]. Diabetic patients and immuno-compromised patients may get

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candida infection[3]. The gram-positive organisms that can cause UTI to include *Staphylococcus aureus, Coagulase Negative Staphylococci, Streptococcusfaecalis, Streptococcus pyogenes*and other streptococci[4]. Urinary tract infection is a common problem faced in practice. It accounts for 35% of all hospital-acquired infections and 2nd most common cause of bacteremia in indoor cases[5,6].

UTI is often treated with different broad-spectrum antibiotics. Antimicrobial resistance to third-generation cephalosporins is increasing. Resistance to β-lactam antibiotics is through the production of the β-lactamase enzyme that breaks down the structure of β- lactam ring of penicillin and other antimicrobials of similar structure. In mid1980's a new type of β-lactamase was produced that could hydrolyse 3rd generation cephalosporins called as Extended-spectrum β-lactamase. (ESBL)[7]. The activity of the majority of ESBL's is inhibited by β-lactamase inhibitors like clavulanic acid, sulbactam, tazobactam. Amp C β-lactamases are cephalosporinases that are poorly inhibited by clavulanic acid. Amp C β-lactamases are presumed to be plasmid-mediated and chromosomal or inducible[8,9].

Increased emergence of antimicrobial resistance in uropathogenic probably due to empirical administration of antibacterial therapy, even before the availability of urine culture reports is a major concern world-wide[10].

By considering all above points, the present study was carried out in our hospital to find out antibiotic sensitivity of gram-negative bacilli causing UTI and also to find out the prevalence of Amp C β-lactamase producing gram-negative bacilli isolated from urine samples of patients with urinary tract infections.

Aim

To study antimicrobial sensitivity of gram-negative bacilli isolated from urine in patients with urinary tract infections and also to find out the prevalence of Amp C β- lactamase producing gram-negative bacilli (GNB) in UTI.

Objectives

1) To isolate and identify gram-negative bacilli from urine samples collected from patients with UTI.
2) To evaluate the antimicrobial sensitivity pattern of gram-negative bacilli (GNB) isolated from urine samples from patients of UTI.
3) To find the prevalence of Amp C β- lactamase producing GNB from urine samples in these patients.

MATERIAL AND METHODS

This study was done in Dr D.Y. Patil Medical College, Hospital and Research Institute, Kolhapur. Duration of the study was for a period from 1st January 2015 to 30th September 2015. Ethical clearance was taken from the institutional ethics committee. Informed consent was taken from all the patients who participated in the study. The study was done with inclusion and exclusion criteria as follows-

Inclusion criteria

1) The study includes all the patients having suspected urinary tract infection.
2) Gram-negative bacilli isolated from urine samples with significant bacteriuria.

Exclusion criteria

1) Urine samples with insignificant bacteriuria.
2) rine samples of significant bacteriuria from which gram-positive bacteriaand Candida spp. were isolated.

Collection of samples- Urine samples were collected from the patients, with suspected urinary tract infection referred for urine culture by clinicians. Mid-stream urine samples were collected in wide mouth sterile bottle by clean catch technique. In women, the periurethral area and perineum was cleaned with soap and water using forward to back technique. In male patients, cleaning of urethral meatus before voiding urine was done.

Processing of samples was done without delay. Samples were plated on Blood Agar, Mac-Conkey Agar and incubated at 37°c for 24 hours. Urine culture was done using semi-quantitative culture technique using standard calibrated loop[2]. Gram staining was done. Gram-negative bacilli were identified by standard bacteriological techniques. Culture showing $\geq 10^5$organisms was reported as significant bacteriuria.

Antimicrobial susceptibility testing of these gram-negative bacilli was performed by Kirby- Baur Disk diffusion method using Mueller Hinton Agar (MHA). Antibiotics disks (from Hi-Media Mumbai) were used. Antibiotics used - Ceftazidime (30µgm), Ceftriaxone (30µgm), Cefoxitin (30µgm), Cefotaxime (30µgm), Ceftazidime- clavulanic acid (30/10µgm), Piperacillin -tazobactam (100/10µgm), Amikacin (30µgm), Nitrofurantoin (300 µgm), Ciprofloxacin (5µgm), Gentamicin (10µgm), Imipenem (10µgm), Meropenem (30µgm). Results were recorded as per guidelines by Clinical Laboratory Standards Institute (CLSI) [11].

GNB showing resistance to 3rd generation cephalosporins were further tested for Amp C β- lactamase production. Presumptive identification Amp C β- lactamase producers was done by-

1) Isolates showing decreased susceptibility to combination disk of Ceftazidime and β- lactamase inhibitors like clavulanic acid.
2) Cefoxitin resistance[12]- Lawn culture of test isolate (0.5 McFarland Std.) was done on Mueller Hinton Agar plate. Cefoxitin disk (30µgm) was placed on inoculated MHA plate. Overnight incubation of plate at 35ºC was done. Isolates showing zone of inhibition of Cefoxitin

disk less than 18 mm were considered as screen positive.

Confirmation of Amp C β- lactamase producing isolates was done using Amp C Disk test[12,13].

Amp C Disk Test[12,13,14]- A lawn culture of std. strain *E.coli* ATCC 25922 was done on MHA plate. A sterile filter paper disk (6mm in diameter) was moistened with sterile distilled water (20µl). This filter paper disk was inoculated with several colonies of the test organism. A Cefoxitin disk (30µgm) was placed on the inoculated plate. Above mentioned moistened filter paper disk was placed beside Cefoxitin disk (Almost touching) The plate was incubated overnight at 37ºC. Results were interpreted as follows-

1) Positive- If there is flattening or indentation of the zone of inhibition of Cefoxitin in the vicinity of test disk, the result is taken as positive.
2) Negative- Result is interpreted as negative if there is an undistorted zone of inhibition of Cefoxitin disk.

Hi Comb MIC Test- Minimum Inhibitory Concentration (MIC) was tested for all Amp C β- lactamase producing isolates using Hi Comb MIC strips of Ceftazidime. (*E.coli* ATCC 25922 was used as negative control)

Data analysis was done by MS -Excel computer language. (Data analysis tool park option). MIC strips and antibiotic disks manufactured by Hi Media Mumbai were used.

Results- Total 288 urine samples obtained from the patients of suspected UTI, were processed in the present study.

Table 1: Prevalence of growth of organisms in urine culture

Total no. of urine samples	Positive Growth	No Growth
288	214 (74.31%)	74 (25.69%)

Out of 214 culture-positive samples, 167 samples showed significant bacteriuria.

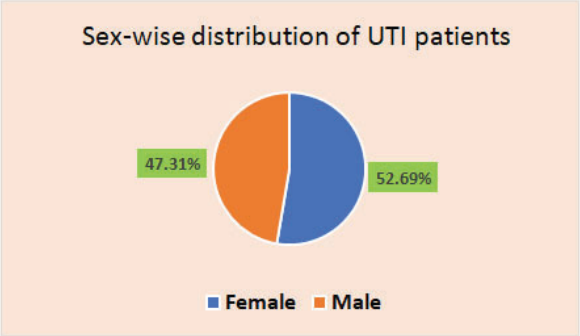

Figure 1: Sex wise distribution of patients with Urinary Tract Infection.

Out of 167 samples of significant bacteriuria, gram-negative bacilli were 125 in number (74.85%), gram-positive bacterial isolates were 38 (22.75%) and candida 4 isolates (2.40%).

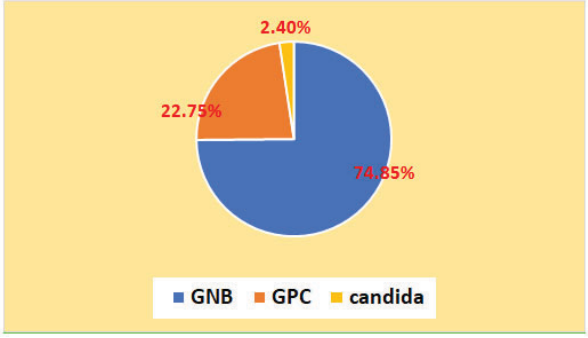

Figure 2: Distribution of bacterial isolates of UTI.

Table 2: Distribution of gram-negative bacilli in UTI

Name of bacteria(n=125)	Number of isolates	Percentage
Escherichia coli	83	66.4%
Citrobacter spp.	19	15.2%
Klebsiella pneumoniae	09	7.2%
Pseudomonas aeruginosa	11	8.8%
Proteus spp.	03	2.4%
Total isolates	125	100%

Table 3: Antibiotic sensitivity pattern of GNB isolated from UTI

Name of antibiotic	*E. coli* (n=83)	Citrobacter spp. (n=19)	Klebsiella pneumoniae (n=09)	P.aeruginosa N=11	Proteus spp. (n=03)
Amikacin	66 (79.51%)	16 (84.21%)	7 (77.78%)	7(63.63%)	2 (66.67%)
Ceftazidime	56 (67.45%)	10 (52.63%)	3(33.33%)	6(54.55%)	1 (33.33%)
Ceftriaxone	50 (60.24%)	12 (63.16%)	4(44.44%)	6(54.55%)	1 (33.33%o
Cefotaxime	52 (62.65%)	11 (57.89%)	3(33.33%)	7(63.63%)	2 (66.67%)

Table 3: *(Continued)*

Name of antibiotic	*E. coli* (n=83)	Citrobacter spp. (n=19)	Klebsiella pneumoniae (n=09)	P.aeruginosa N=11	Proteus spp. (n=03)
Ceftazidime- clavulanic acid	63 (75.90%)	11 (57.89%)	6(66.67%)	8(72.73%)	2 (66.67%)
Piperacillin-tazobactam	68 (81.92%)	11 (57.89%)	7(77.78%)	8(72.73%)	1 (33.33%)
Imipenem	79 (95.18%)	15 (78.95%)	7(77.78%)	2(18.18%)	2 (66.67%)
Meropenem	76 (91.57%)	14 (73.68%)	8(88.89%)	3(27.27%)	3 (100%)
Nitrofurantoin	67 (80.72%)	16 (84.21%)	7(77.78%)	6(54.55%)	2 (66.67%)
Ciprofloxacin	62 (74.70%)	14 (73.68%)	6(66.67%)	6(54.55%)	2 (66.67%)
Gentamicin	51 (61.45%)	12 (63.16%)	6(66.67%)	7(63.63%)	2 (66.67%)

Table 4: Gram negative isolates positive for Amp C β-lactamase production by confirmatory test.

Name of bacteria	Number of isolates	Percentage
Escherichia coli(n=83)	17	20.48%
Citrobacter spp.(n=19)	6	31.58%
Klebsiella pneumoniae (n=09)	3	33.33%
Pseudomonas aeruginosa(n=11)	0	00%
Proteus spp. (n=03)	1	33.33%
Total (n=125)	27	21.6%

Out of total 125gram negative bacilli, 27 isolates were confirmed for Amp C β-lactamase production by Amp C disk test (21.6%). Minimum Inhibitory Concentration (MIC) was tested in these isolates by Hi-comb MIC strips.

Table 5: Minimum Inhibitory Concentration (MIC) of ceftazidime in Amp C producers

MIC of Ceftazidime in (µgm/ml)	Isolates of *E.coli* (n= 17)	Isolates of Citrobacter spp. (n=6)	Isolates of Klebsiella spp. (n=3)	Isolates of Proteus spp. (n=1)
2	-	-	-	-
4	3	1	-	-
8	-	-	-	-
16	2	1	-	-
32	3	2	-	-
64	7	1	2	-
128	1	1	1	1
256	1	-	-	-

Table 6: Sensitivity of Amp C β-lactamase producing organisms to different antibiotics

Name of bacteria	*E.coli* (n=17)	Citrobacter spp. (n=6)	Klebsiella spp.(n=3)	Proteus spp. (n=1)
Ceftazidime	3 (17.65%)	1(16.67%)	-	-
Ceftriaxone	2(11.76%)	-	-	-
Cefotaxime	2(11.76%)	1(16.67%)	1 (33.33%)	1(100%)
Amikacin	13(76.47%)	4(66.67%)	2(66.67%)	1(100%)
Piperacillin-tazobactam	5(29.41%)	3(50%)	1(33.33%)	-
Ceftazidime-clavuanic acid	-	-	-	-
Imipenem	16(94.12%)	5(83.33%)	3 (100%)	1 (100%)
Meropenem	16(94.12%)	6 (100%)	2(66.67%)	-
Nitrofurantoin	14(82.35%)	4(66.67%)	2(66.67%)	1 (100%)
Ciprofloxacin	12(70.59%)	3(50%)	2(66.67%)	-
Gentamicin	11(64.71%)	4 (66.67%)	1(33.33%)	1 (100%)

DISCUSSION

Urinary tract infection is the most common infection in human being. Bacteria causing UTI have become resistant to many broad-spectrum antibiotics. Indiscriminate use of antibiotics in the treatment of UTI has resulted in resistant organisms. By keeping all this in mind, the present study was carried out to find out gram-negative bacilli causing UTI and antibiotic susceptibility testing of these isolates.

In the current study, a total of 288 urine samples from suspected UTI were processed. Out of which 74 samples were culture-negative (i.e. 25.69%). From 214 culture-positive samples, 167 samples showed significant bacteriuria (78.03%). Female patients were 88 in number (52.69%) and male patients were 79 in number (47.31%). Similar results were reported by Sayd Mushtaq Ahmed, Ramkrishna Pai et al in their study, which showed 67.12% urine samples from UTI were culture positive in female patients[15]. Another study by K.D.Deshpande et al. in 2011 showed UTI was common in female (59.2%) in their study[16]. B. Shanthi et al. also showed the prevalence of UTI high among female i.e. 58.18% and 41.8% in the male in their study[17]. UTI is common in female due to following reasons[2] -1) short female urethra as compared with male 2) proximity of the female urethra to the anus and moist periurethral area 3) hormonal changes and anatomical differences 4) pregnancy predisposes to UTI[2].

In the present study, out of 167 samples of significant bacteriuria, 125 gram-negative bacilli (GNB) were isolated (i.e. 74.85%). Most common isolate among gram-negative bacilli was *E.coli* (83/125 i.e. 66.4%), followed by *Citrobacter spp*. (19/125 i.e. 15.2%). A study by Niranjan V and Malini A From Puducherry India, in their study, showed *E.coli* as most common isolate accounting for 56.8%[18]. Another study by S.Dugal et al. from Mumbai showed in his study, *E.coli* as most common isolate (80%), other isolates were *Klebsiella pneumoniae* (16.07%), *P.aeruginosa* (2.67%), and *Proteus spp*.(0.8%)[19]. Sayd Mustq et al. in his study reported *E.coli* (81.80%) as a predominant organism in UTI, followed by *K.pneumoniae* 14.87%, *P.aeruginosa* 2.74%, *Citrobacter spp*. 0.59%[15].

The present study showed resistance of *E.coli* isolates to 3rd generation cephalosporins like Ceftazidime, Cefotaxime, Ceftriaxone. The sensitivity of these isolates to Ceftazidime-clavulanic acid was 75.90% and resistance was 24.10%. Susceptibility of *E.coli* isolates to imipenem was 95.18%, meropenem 91.57% and piperacillin-tazobactam was 81.92%. Susceptibility of these isolates to nitrofurantoin was 80.72%, amikacin 79.51% and ciprofloxacin 74.70%. A study by Sayd Mushtaq et al.[15] showed in their study, high resistance of gram-negative bacilli to cefuroxime, ceftriaxone and Ciprofloxacin. In their study, antibiotic sensitivity of *E.coli*showed high susceptibility pattern towards imipenem (98.5%), nitrofurantoin and piperacillin-tazobactam (91.5%). Another study by Niranjan V and Malini A showed *E.coli* isolates were sensitive to amikacin 82,6%, nitrofurantoin 82.1%, and imipenem 98.9% [18].

The current study showed a sensitivity of *Citrobacter* isolates to amikacin84.21%, nitrofurantoin 84.21%, imipenem 78.95% and meropenem 73.68%. In my study, these isolates showed less sensitivity to ceftazidime (52.63%), cefotaxime (57.89%)and ceftriaxone (63.16%). Also, the reduced sensitivity of these isolates was present to ceftazidime-clavulanic acid 57.89% and piperacillin-tazobactam 57.89% and gentamicin 63.15%. A study by K. D. Deshpande et al.[16] from Latur showed sensitivity pattern of *Citrobacter* isolates asamikacin 74%, nitrofurantoin 77.7%, imipenem 100%. Their study also showed reduced sensitivity of these isolates to ceftazidime 40.7%, cefotaxime37% and ceftriaxone 40.7% [16]. A study by Dr Durgesh Mahajan et al from Yavatmal, Maharashtra, showed a sensitivity of *Citrobacter* isolates

to amikacin 100%, nitrofurantoin 75%, imipenem 75% and gentamicin 75%[19]. Their study also reported reduced susceptibility of Citrobacter isolates to ceftazidime 50% and ciprofloxacin 50% [20].

The present study showed less sensitivity of *K.pneumoniae* to ceftazidime (33.33%), ceftriaxone (44.44%), cefotaxime (33.33%). These isolates also showed less sensitivity to ceftazidime-clavulanic acid (66.67%), ciprofloxacin(66.67%) and gentamicin (66.67%). Sensitivity of these isolates to meropenem(88.89%) , imipenem(77.78%) and nitrofurantoin (77.78%) was good. A study by K.D. Deshpande et al showed less sensitivity of *K.pneumoniae*to ceftazidime (31.7%), ceftriaxone (33.33%), cefotaxime (35.7%)[16]. In their study, these isolates were 100% sensitive to imipenem[16]. A study by Monika Yadav et al from Manipur, India in their study showed susceptibility of *Klebsiella spp.*to imipenem (89.4%), nitrofurantoin 54.3%[21]. The study also reported resistance of these isolates to ceftazidime. Their study also reported resistance of *Proteus* isolates to ceftazidime (12/22 isolates resistant)and sensitivity to imipenem 95.4%[21]. In the present study, *Proteus* isolates were resistant to 3rd generation cephalosporins and sensitivity to imipenem was 66.67% but for meropenem was 100%. The present study showed reduced susceptibility of *Pseudomonas aeruginosa* isolates to ceftazidime (54.55%), ceftriaxone (54.55%). These isolates were sensitive to ceftazidime-clavulanic acid (72.73%), piperacillin-tazobactam (72.73%). These isolates showed less sensitivity to imipenem (18.18%) and meropenem (27.27%). This resistance of *Pseudomonas* isolates to imipenem and meropenem may be due to metallo-β- lactamase production. A study by Monika Yadav, Rohan Pal et al in their study reported susceptibility of *Pseudomonas* isolates to ceftazidime (33.33%), ceftriaxone (29.1%), piperacillin-tazobactam (66.6%), but sensitivity to imipenem was 91.6%[21].

Indiscriminate use of antibiotics, inadequate intake of antibiotics results in the emergence of resistant organisms which increases morbidity and mortality. Antibiotic resistance is a worldwide problem. In the present study, organisms which were resistant to 3rd generation cephalosporins and ceftazidime -clavulanic acid were tested for Amp C β-lactamase production. The screening was done by cefoxitin resistance test and confirmation was done by Amp C Disk Test. In the present study, Amp C β-lactamase producing gram-negative bacilli isolated from UTI were 21.6%. MIC of these isolates ranged between 4µgm/ml – 256 µgm/ml. Amp C producing organisms were sensitive to imipenem, meropenem and nitrofurantoin. A study by Pottahil Shinu, R. Bareja et al. showed in their study prevalence of Amp C β-lactamase production in gram-negative bacilli from UTI 21.26% [22]. Ephram Ibadan et al. in his study showed Amp C β-lactamase production in UTI 15.1% [23]. Another study by Anandhi, Lakshmanan et al. showed Amp C production in *E.coli* isolates from urine 52.38% by disk diffusion method[24]. One more study by Anitha Madhavan et al. from Kerala reported Amp C β-lactamase production in UTI 41.42%[25].

80-85% of urinary tract infections are due to gram-negative bacilli while 15-20% are due to gram-positive bacteria[26]. Prevalence of drug resistance of microorganisms in UTI is different in different environmental conditions [27]. Extended-spectrum β – lactamase (ESBL) and Amp C β-lactamase producing gram-negative bacilli is a major problem in healthcare. Different phenotypic and genotypic methods are used to detect ESBL and Amp C β-lactamase. In the current study, simple phenotypic methods like cefoxitin resistance test and Amp C Disk test were used. They are easy to perform and interpret. Regular reporting of Amp C β-lactamase producing organisms is useful to the clinician to select proper antibiotic.

CONCLUSION

Present study concludes grey negative bacilli (74.85%) were common organisms responsible for urinary tract infection (UTI). *E.coli* (66.4%) was the most predominant causative agent of UTI.Resistance was noted by GNB to 3rd generation cephalosporins. Gram-negative bacilli showed susceptibility to amikacin, nitrofurantoin, imipenem and meropenem. *Pseudomonas* isolates showed resistance to imipenem and meropenem.Prevalence of Amp C β-lactamase producing GNB in UTI was 21.6%. Amp C β-lactamase producing organisms were susceptible to imipenem, meropenem and nitrofurantoin. Regular reporting of antimicrobial resistance mediated through Amp C β-lactamase producing organisms, judicious use of antibiotics, effective hand wash is very useful to prevent the emergence of antimicrobial resistance.

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