Anti-Microbial Susceptibility Patterns of Enterobacteriaceae Isolated From A Tertiary Care Unit In Gujarat

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Citation

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Abstract

Objectives: Present study was undertaken to assess antibiotic susceptibility patterns of Enterobacteriaceae at a tertiary care hospital in Gujarat, India.
Methods: Out of 276 culture positive samples, 154 samples of Enterbacteriaceae were examined and 11 different types of specimen were collected. Microbial sensitivity testing was done using disk diffusion test with Escherichia coli NCTC 10418 as per CLSI guidelines.
Results: Highest Enterobacteriacae infections were found in urine followed by pus and sputum. Enterobacteriacae species demonstrated marked resistance against monotherapy of penicillins and cephalosporins. Combination of ampicillin, amoxicillin and third generation cephalosporins with sulbactam and monotherapy of amikacin showed higher sensitivity to Enterobacteriacae infections but maximum sensitivity was shown by carbapenems.
Conclusions: Urinary tract infection was the most common hospital acquired infection. Co-administration of -lactamase inhibitor markedly expanded the anti-microbial sensitivity of penicillins and cephalosporins. Use of amikacin and carbapenems should be restricted to severe nosocomial infections to avoid rapid emergence of resistant strains.

INTRODUCTION

The development of antibiotic resistance can be viewed as a global problem in microbial genetic ecology. It is a very complex problem to contemplate, let alone solve, due to the geographic scale, the variety of environmental factors, and the enormous number and diversity of microbial participants. Extended spectrum -lactamases (ESbLs) continue to be a major problem in clinical setups worldwide, conferring resistance against extended spectrum cephalosporins. Increasing resistance to third and fourth generation cephalosporins has become a cause of concern especially amongst Enterobacteriaceae family which is one of the main cause of nosocomial infections. ESbLs are the derivatives of common b-lactamases (TEM and SHV b-lactamases) that have undergone one or more amino acid substitutions near the active site of the enzyme, thus increasing their affinity and the hydrolytic activity against third generation cephalosporins and monobactams. Extensive use of newer generation cephalosporins has been the strong factor for the evolution of newer b-lactamases such as ESbLs. ESbLs are encoded by transferable conjugative plasmids, which often code resistance determinants to other antimicrobial agents such as aminoglycosides. These conjugative plasmids are responsible for the dissemination of resistance to other members of gram negative bacteria in hospitals and in the community ([1,2,3,4]).

Bacterial infection is the most common cause for hospital visits. In almost all cases of nosocomial infection, there is a need to start treatment before the final microbiological results are available. Area-specific monitoring studies aimed to gain knowledge about the type of pathogens responsible for specific infection and their resistance patterns may help the clinicians to choose the correct empirical treatment. Hence, this study was undertaken to find out the antibiotic susceptibility pattern of the isolated pathogenic Enterobacteriaceae from various specimens from hospital acquired infections.

MATERIALS AND METHODS

Our study group comprised of patients who were clinical suspects of bacterial infection or who had undergone various surgical procedures. Project was undertaken at Rajasthan

Hospitals, Ahmedabad, Gujarat, India. A protocol was designed and all the information pertaining to the patient's name, age, sex and culture susceptibility etc. were recorded on individual basis. Collected specimen comprised of urine, pus, sputum, endotracheal secretion (ET), blood, bile, broncho-alveolar lavage (BAL), semen, body tissues and various body fluids. All the samples were collected by standard procedures in aseptic closed containers and extensive care was taken to avoid contamination such as in case of urine sample, standard “clean-catch” method was adopted. Specimen was sent to laboratory as early as possible. If there was a delay of more that two hours, it was refrigerated except in suspected cases of infection with H. influenza, S. pneumoniae and Nisseriae groups, as they are susceptible to low temperature ([5]). The samples collected were examined microscopically for pus, epithelial and blood cells. The samples were also processed using standard microbiological procedures. All the samples were processed on blood agar and MacConkey's medium followed by inoculation by four flame streak method. Antibiotic susceptibility was confirmed by disk diffusion technique on Muller-Hinton medium (Becton Dickinson Microbiological Systems, Cockysville, MD) according to Clinical Laboratory Standard Institute (CLSI) guidelines ([6]). ZN stain was performed to find out the acid fast organisms. Paper disks (Hi-media, Mumbai) were impregnated with antibiotics (Sigma Chemical Co., St. Louis, Mo.): Penicillins: Ampicillin (10mcg), Amoxycillin (20mcg), Cephalosporins: Cefoperazone (75 mcg), Cefotaxime (30mcg), Ceftriaxone (30mcg), Combinations: Ampicillin + Sulbactum (10/10 mcg), Amoxycillin + Clavulinic acid (20/10 mcg), Cefoperazone + Sulbactum (75/10 mcg), Cefotaxime + Sulbactum (30/10 mcg), Ceftriaxone + Sulbactum (30/10 mcg), Carbepenems: Imipenem (10mcg) , Meropenem (10 mcg), Monobactums: Aztreonam (30 mcg), Aminoglycosides: Amikacin (30 mcg), Netilmycin (30 mcg) Quinolones: Ciprofloxacin (5mcg), Gatifloxacin (5mcg).

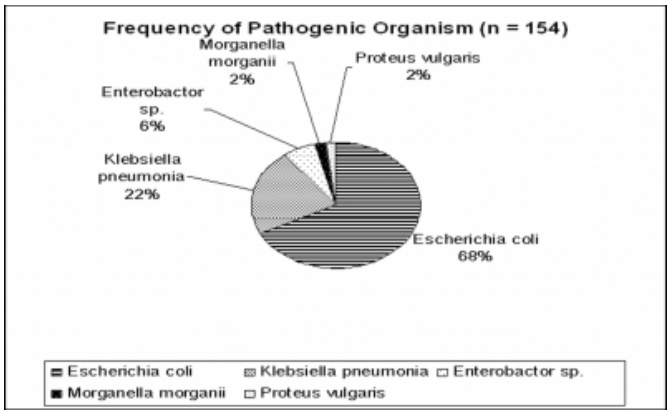
Results were interpreted as sensitive (S), intermediate resistant (I) or resistant (R) based on CLSI guidelines ([6]). The category “susceptible” was defined as identification of a strain as susceptible by the disk diffusion method. Quality control strain of Escherichia coli (E. coli) – NCTC – 10418 was used to validate the results of the antimicrobial discs. Susceptibility data were compared by using a Chi-square test and one-way ANOVA with SPSS software for Windows, version 12. Both susceptibility and resistance were calculated as percentages with 95% confidence intervals. The analysis was performed on the cross-tabulated values of the presence of the resistant/intermediate/susceptible isolates, according to the categories of the selected variable. A p-value of <0.05 was considered to be statistically significant.

RESULTS

572 samples were studied, out of which, 276 (48.35%) were culture positive samples. From this culture positive samples, 154 samples (55.80%) were from patients infected with Enterobacteriacea, and among them, 93 (60.39%) were from males while 61 (39.61%) specimen were from females. In the present study, highest causative organism isolated was E. coli (67.53%), followed by Klebsiella pneumoniae (K. pneumoniae) (22.08%), Enterobacter sp. (6.49%), Morganella morganii (M.morganii) (1.95%) and Proteus vulgaris (P.vulgaris) (1.95%) (Figure 1).

Figure 1

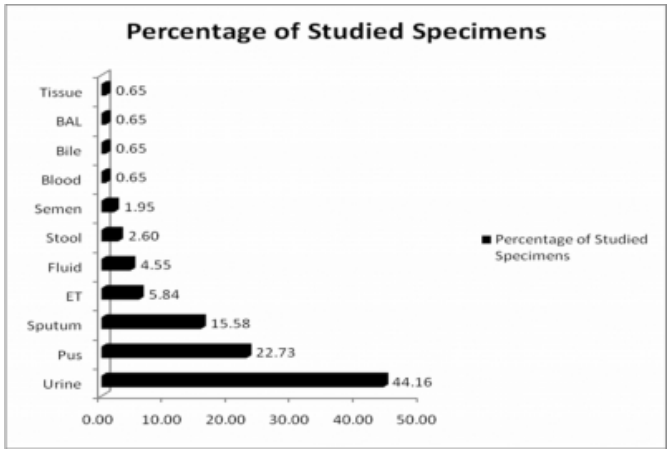
Figure 1: Frequency and percentage of pathogenic organisms (bacteria) (n = 154) isolated in present study



Urine (44.16%) was the specimen source for majority of isolates, followed by pus (22.73%) and sputum (15.58%) (Figure 2).

Figure 2

Figure 2: Frequency and percentage of specimens (bacteria) (n = 154) studied in the present study (BAL:broncho-alveolar lavage; ET:endotracheal secretion)



Frequency of isolation of pathogenic organisms from various specimens is depicted in Table–1. It can be observed that with urine as the specimen source, highest infective organism isolated was E. coli (76.47%) followed by K. pneumoniae (13.24%), Enterobacter sp. (8.82%), and P. vulgaris (1.47%). In pus, highest infective organism isolated was E. coli (57.14%), followed by K. pneumoniae (17.14 %), Enterobacter sp. (11.43%). M morganii (8.57%) and P. vulgaris (5.71%). In sputum, only two organisms, K. pneumoniae (54.17%) and E. coli (45.83%), were isolated. Most incidences of respiratory tract infections were attributed to K. pneumoniae in sputum. In ET secretion, E. coli was found to be the principal organism isolated (88.89%), followed by K. pneumonia (11.11%). E. coli (75.00%) and K. pneumoniae (25.00%) were isolated from semen and stool specimen respectively. In various body fluids, most common infective organism found was E. coli (71.43%) followed by K. pneumoniae (28.57%). K. pneumoniae was the only organism isolated from a single blood specimen in our study. Similarly, E. coli was the only organism isolated from various body fluids (BAL, tissue, bile etc.).

Figure 3

Table: 1 Specimen wise frequency of isolation of pathogenic organisms (bacteria) from specific sites. (Values shown are percentage of total) (n = 154)

Specimen		Organisms					
		E. coli	*K. pneumoniae*	*Enterobacter sp.*	*M. morganii*	*P. vulgaris*	*Total (Nos. of Samples)*
Urine	Nos.	52	9	6	0	1	68
	%	76.47	13.24	8.82	0.00	1.47	100
Pus	Nos.	20	6	4	3	2	35
	%	57.14	17.14	11.43	8.57	5.71	100
Sputum	Nos.	11	13	0	0	0	24
	%	45.83	54.17	0.00	0.00	0.00	100
ET	Nos.	8	1	0	0	0	9
	%	8.89	11.11	0.00	0.00	0.00	100
Fluid	Nos.	5	2	0	0	0	7
	%	71.43	28.57	0.00	0.00	0.00	100
Stool	Nos.	3	1	0	0	0	4
	%	75.00	25.00	0.00	0.00	0.00	100
Semen	Nos.	2	1	0	0	0	3
	%	66.67	33.33	0.00	0.00	0.00	100
BAL	Nos.	1	0	0	0	0	1
	%	100	0.00	0.00	0.00	0.00	100
Blood	Nos.	0	1	0	0	0	1
	%	0.00	100	0.00	0.00	0.00	100
Tissue	Nos.	1	0	0	0	0	1
	%	100	0.00	0.00	0.00	0.00	100
Bile	Nos.	1	0	0	0	0	1
	%	100	0.00	0.00	0.00	0.00	100

SUSCEPTIBILITY PATTERNS

All antibiotics used in the present study as monotherapy, did not demonstrate statistically significant susceptibility patterns (P>0.05) with the exception of imipenem and meropenem. Although aztreonam has been reported to have strong activity against gram-negative bacteria, the study revealed high levels of resistance (30% to 100%). Table 2 and figures 3-7 show the overall results of susceptibility pattern of E. coli, K. pneumoniae, P. vulgaris , M. morganii and Enterobacter sp.

Figure 4

Table 2: Antibiotic resistance patterns against monotherapy antibiotics. (Values shown are percentage of total) (S:Sensitive; I:Intermediate resistant; R:Resistant)

Antimicrobial	Species														
	E. coli			*K. pneumoniae*			*M. morganii*			*P. vulgaris*			*Enterobacter sp.*		
	Percentage (%)			Percentage (%)			Percentage (%)			Percentage (%)			Percentage (%)		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Imipenem	99.04	0	0.96	100	0	0	100	0	0	100	0	0	100	0	0
Meropenem	98.08	0.96	0.96	100	0	0	100	0	0	100	0	0	100	0	0
Aztreonam	13.46	0	86.54	20.59	2.94	76.47	33.33	33.33	33.33	0	0	100	20	0	80
Amikacin	69.23	14.42	16.35	76.47	8.82	14.71	33.33	66.67	0	0	0	100	60	0	40
Netilmycin	39.42	9.62	50.96	52.94	11.76	35.29	66.67	0	33.33	0	0	100	60	0	40
Ciprofloxacin	3.85	0.96	95.19	26.47	8.82	64.71	66.67	0	33.33	0	0	100	40	0	60
Gatifloxacin	15.38	8.65	74.04	38.24	2.94	58.82	66.67	33.33	0	66.67	0	33.33	80	20	0

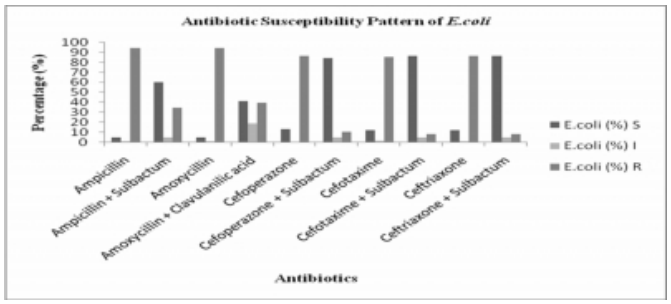
E. COLI

We observed that E.coli was highly sensitive to carbapenem group of antibiotics like imipenem (99.04%) and meropenem (98.08%) while aztreonam showed 86.54% resistance (p<0.001). Ampicillin-sulbactum, cefoperazone-sulbactum, cefotaxime-sulbactum and ceftriaxone-sulbactum combinations had significantly greater antibacterial activity

against E.coli when compared to their respective monotherapies (p<0.001). Other antibiotics studied presented susceptibility rates of less than 50%. (Figure 3)

Figure 5

Figure 3: Antibiotic susceptibility pattern of against monotherapy vs. combination therapy (S:Sensitive; I:Intermediate resistant; R:Resistant)

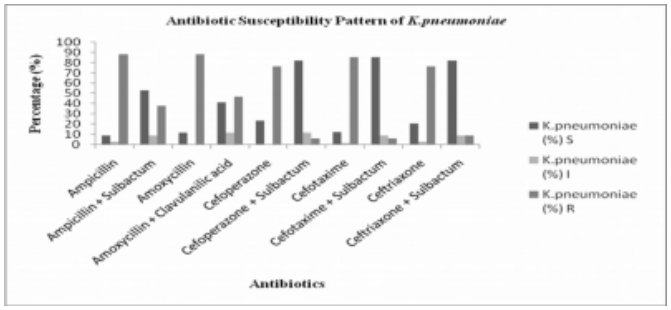


K. PNEUMONIAE

Among K. pneumoniae (n = 34) isolates, all samples were cent percent susceptible to imipenem and meropenem. Susceptibility rates to amikacin and netilmycin were 76.47% and 52.94% respectively. Susceptibility rates were significantly greater (p<0.001) for cefotaxime-sulbactum and ceftriaxone-sulbactum combinations (85.29% and 82.35% respectively). All remaining antimicrobials demonstrated considerably lower susceptibility rates (<55%). (Figure 4)

Figure 6

Figure 4: Antibiotic susceptibility pattern of against monotherapy vs. combination therapy (S:Sensitive; I:Intermediate resistant; R:Resistant)

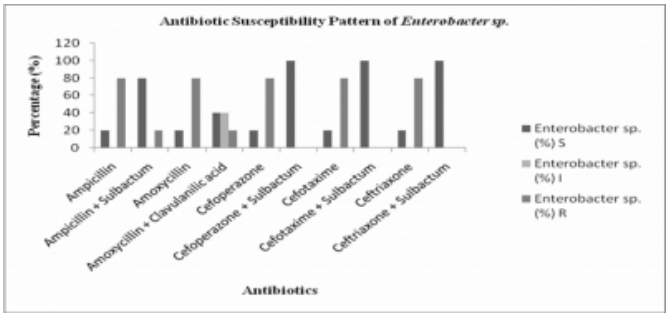


ENTEROBACTER SP.

All enterobacter species (n=10) isolates were 100% susceptible to imipenem and meropenem. While susceptibility rates to amikacin, netilmycin and gatifloxacin were 60%, 60% and 80% respectively. Susceptibility to cephalosporin was only 20% due to ESbLs production in 80% specimens. However, enterobacter sp. were 100% susceptible to cephalosporins-sulbactum combinations (p<0.001). (Figure 5)

Figure 7

Figure 5: Antibiotic susceptibility pattern of against monotherapy vs. combination therapy (S:Sensitive; I:Intermediate resistant; R:Resistant)



M. MORGANII AND P. VULGARIS

M. morganii and P. vulgaris (n = 3 each) isolates, were totally susceptible to imipenem and meropenem. M. morganii was 100% susceptible to monotherapy of cefoperazone also. On the other hand, Combination of ampicillin, cefotaxime and ceftriaxone with sulbactum demonstrated 100 % susceptibility against M. Morganii, but not against P. vulgaris isolates. (Figures 6,7)

Figure 8

Figure 6: Antibiotic susceptibility pattern of against monotherapy vs. combination therapy (S:Sensitive; I:Intermediate resistant; R:Resistant)

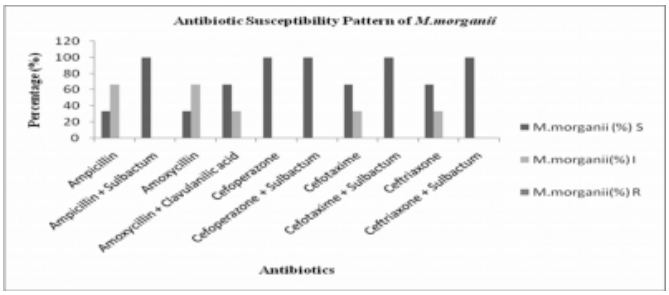
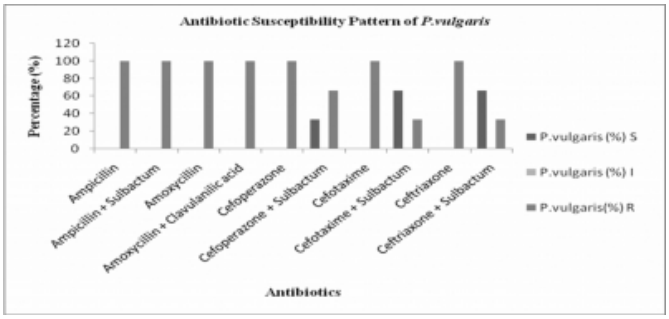


Figure 9

Figure 7: Antibiotic susceptibility pattern of against monotherapy vs. combination therapy (S:Sensitive; I:Intermediate resistant; R:Resistant)



DISCUSSION

Out of 154 cultures positive specimen isolates, 44.16% (68 specimens) were urine samples indicating that urinary tract infection (UTI) is the most common hospital acquired infection. A high isolation rate of pathogens from urine samples of clinically suspected UTI show a good correlation between clinical findings and microbiological methods. UTI is one of the most important causes of morbidity in general population and is the second most common cause of hospital visits. The incidence of UTI is greater in women than men, which may be either due to anatomical predisposition or urolithial mucosal adherence to mucopolysaccharide lining or other host factors ([7] , [8]). Gram negative organisms were the commonest organisms present in specimens, amongst which E. coli was the principal urinary pathogen. Data presented in this study indicated that E. coli infection predominated in the urine (76.47%) followed by pus (57.14%) and all other specimen sites (Table 1). Other organisms isolated from urine were K. pneumoniae (13.24%), Enterobacter sp. (8.82 %) and P. vulgaris (1.47%). These frequencies of isolation of various urinary pathogens are consistent with the reports of the recently published studies ([9,10,11]).

It should be noted that, at least 66.89% of samples in the current study were from clinically significant sources (urinary tract and pus). On the other hand, 21.42% of the samples were from the respiratory tract. The frequency and percentage of specimen distribution in our study closely reflects the prevalence of gram negative bacteria in similar settings reported elsewhere ([12,13,14]).

Penicillin group of antibiotics are drugs of choice for a wide variety of infectious diseases. Unfortunately these drugs are readily hydrolyzed by broad spectrum β-lactamases that are found with increasing frequency in clinical isolates of these gram negative bacteria. Many reports in the literature suggest an increased resistance to ampicillin (80-100%). A study from western Nepal reported high prevalence of resistance to ampicillin, nalidixic acid and norfloxacin ([12] , [13]). However, concurrent administration of a β-lactamase inhibitor such as clavulanate or sulbactum markedly expands the spectrum of activity of these drugs. The susceptibility of all gram negative bacteria significantly increased and ranged from 50% to 80% (Table 2 and Figures 3-7). Thus it is evident that β-lactamase producing gram negative organisms are resistant to monotherapy of penicillin.

Our results with cephalosporins are in corroboration with the one reported by other workers ([14,15,16,17]) that the overall resistance to various generations of Cephalosporins was high on account of the production of ESbLs by the bacteria involved. Hence, addition of sulbactum to cefoperazone, cefotaxime and ceftraixone monotherapy significantly reduced the percentage resistance and increased the percentage susceptibility against all the organisms (Table 2 and Figure 3-7). Carbapenems, the antibiotics class resistant to most of the β-lactamases, have a broader spectrum of activity then do other β-lactam antibiotics. It is resistant to hydrolysis by most β-lactamases. Activity is excellent against Enterobacteriaceae including organisms resistant to cephalosporins by virtue of expression of chromosomal or plasmid extended spectrum β-lactamases. Most of the strains of Pseudomonas and Acinetobacter are inhibited by carbapenems. Hence, it would be prudent to use imipenem or meropenem for empirical treatment of serious infections in hospitalized patients.

Aztreonam binds the penicillin-binding proteins of gram-positive and anaerobic bacteria very poorly and is largely ineffective against them. It is known to be effective against a wide range of gram-negative bacteria, however in the present study all organisms were resistant to aztreonam, with P.vulgaris being 100% and Enterbacter sp. being 80% resistant. Sensitivity of amikacin, against E. coli (69.23%) and K. pneumoniae (76.47%) was higher when compared to netilmycin, the latest aminoglycoside marketed (E.coli: 39.42%; K. pneumoniae: 52.94%). Similar observations have been reported by Smitha et al ([18]). Literature states that gatifloxacin (fluroquinolone) is demonstrated to be more active than others. In our study also, it showed improved sensitivity against gram negative pathogens. Our results correlate well with the earlier published reports from India ([19] , [20]).

From present investigation, we can say that there are increasing instances where the resistance to antimicrobials acquired by previously susceptible organisms is the cause of treatment failure, or requires administration of larger doses or more expensive and toxic agents. Irrational and inappropriate use of antibiotics has been a major cause in development of drug resistance. Therapeutic decisions in infections involve consideration of susceptibility-resistance patterns, pharmacokinetic profile, prophylactic/combined antibiotic therapy, host-defense mechanisms, local factors and adverse reactions of the drug etc. There is a need to emphasize the rational use of antimicrobials and strictly adhere to the concept of "reserve drugs" to minimize the misuse of available antimicrobials. In addition, regular antimicrobial susceptibility surveillance is essential for area wise monitoring of the resistance patterns. An effective national and state level antibiotic policy and draft guidelines should be introduced to preserve antibiotic effectiveness, and for better patient management. Industry and government should boost basic research for identifying new and effective molecules to combat the resistant micro-organisms. Physicians and pharmacists should avoid the misuse of potent molecules and reserve such molecules for severely resistant cases only.

CONCLUSIONS

Based on our observations of the present study we recommend use of antimicrobials like ampicillin, amoxicillin and third generation cephalosporins (cefoperazone, cefotaxime and ceftriaxone) in combination with -Lactamase inhibitors (clavulanate or sulbactum) for the treatment of Enterobacteriaceae infections in similar hospital settings. Further imipenem, meropenem and amikacin should be considered as reserved drugs for the treatment of severe nosocomial infections to avoid emergence of resistant strains.

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