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RESEARCH ARTICLE

BACTERIOLOGICAL PROFILE OF GRAM NEGATIVE ORGANISMS AND DRUG SENSITIVITY PATTERN OF ESCHERICHIA COLI IN HOSPITAL SPECIMENS

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ABSTRACT

Among the gram negative organisms Escherichia coli commonly causes both nosocomial and community acquired infections in human. Occurrence of multiresistant strains necessitates periodic monitoring of its susceptibility pattern. This retrospective study was done in the Department of Pharmacology and Microbiology at Sri Manakula Vinayagar Medical College Hospital, Pondicherry. During the period from January 2012 to June 2012, a total of 5381 specimens (Urine, Blood, Pus, Swab, Cerebrospinal fluid etc.) were processed for culture and sensitivity according to CLSI recommendations. Sensitivity pattern was shown using descriptive stastistics. Gram negative bacteria accounted for about 62% of the isolates. The main species were Escherichia coli 483(52.6%), Klebsiella sp.196 (21.3%), Pseudomonas sp.167 (18%), Proteus sp.38 (4%), Salmonella sp.17 (2%),Citrobacter 8 (0.8%), Moraxella 3(0.3%), Vibrio 2(0.2%), and H.influenza, Acinectobacter and Enterobacter 1(0.1%).Escherichia coli showed high level of susceptibility to Imipenem (99.7%), Piperacillin+Tazobactum (97%), Meropenam (95%), Nitrofurantoin (92%) and Amikacin(84%). Very high rates of resistance was seen with Ampicillin(88%), Nalidixic acid (86%), Amoxycillin + clavulanic acid(84%) and Cotrimoxazole(74%). Periodic monitoring of antimicrobial susceptibility both in the community and hospital settings is recommended to identify the sensitivity and resistant patterns of E.coli.

INTRODUCTION

Antimicrobials have transformed our ability to treat many infectious diseases that were killers for many decades. These agents provide the most dramatic examples of the advances of modern medicine. However various microorganisms have survived by their ability to adapt to antimicrobial agents leading to antimicrobial resistance. Importantly gram negative bacterial isolates account for significant proportion of hospital and community associated infections.

Among the gram negative bacteria Escherichia coli(*E.coli*) that belongs to the family Enterobacteriaeceae is the common cause of diarrhoeal diseases, urinary tract infection, neonatal meningitis etc., in humans (Thomas *et al.,* 2008). Increasing rates of resistance among *E. coli* is a growing concern in both developed and developing countries (Bell *et al.,* 2002; El Kholy *et al.,* 2003).The antimicrobial susceptibility profiles of *E. coli* also have showed geographic variations as well as significant differences in various populations and environment (Erb *et al.,* 2007;Von Baum *et al.,* 2000).

In India the reasons for increasing antibiotic resistance could be due to irrational use of antibiotics, over the counter availability of higher antibiotics, high prevalence of infection and poor monitoring of antibiotic susceptibility surveillance in hospitals. Significant studies done in India shown the prevalence and antimicrobial resistance patterns of *E. coli* from various clinical sources (Summiya Mulla *et al.,* 2011). Antibiotic policy of a particular hospital should be based on antimicrobial sensitivity profile of microorganisms and this will be useful guide for empirical treatment. Periodic surveillance and monitoring programs are helpful for the development of empirical approaches for the treatment of serious infections, as well as, prevention and control of infections caused by resistant microorganisms (Deasy 2009; Nicolau 2009). Therefore, the present study was undertaken with the objective of determining the current status of antimicrobial susceptibility pattern of the most common isolate, E. coli from hospital specimens.

MATERIALS AND METHODS

This retrospective analysis was carried out in the Department of Pharmacology and Microbiology at Sri Manakula Vinayagar Medical College Hospital, Pondicherry. The samples received from various outpatient and inpatients between January 2012 to June 2012 were included in the study. Clinical specimens include urine, blood, pus, swabs, cerebrospinal fluid(CSF), ascitic fluid(AF), synovial fluid(SF), pleural fluid(PF), stool, sputum etc., Samples were processed for culture and sensitivity by standard methods.[9] All significant isolates were identified by standard procedures and their antimicrobial susceptibility was tested by Kirby Bauer disc diffusion method and interpreted as per Clinical and Laboratory Standards Institute recommendations (CLSI/NCCLS, 2005). The zone of

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inhibition of organisms growth was measured and interpreted as susceptible and resistant based on CLSI guidelinesand interpreted as susceptible, intermediate or resistant based on CLSI guidelines. Control strains were used for checking the quality of discs. The antibiotics which were included for the isolates were Cotrimoxazole, Ampicillin, Amoxycillin+clavulanic acid, Ticarcillin, Piperacillin, Piperacilline+Tazobactum, Imipenam, Meropenam, Aztreonam, Nalidixic acid, Norfloxacin, Ciprofloxacin, Levofloxacin, Amikacin, Tobramicin, Cefazolin, Cefotaxime, Ceftazidime, Ceftriaxone, Tetracycline and Tigecycline. The datas were entered in Microsoft excel and analyzed using Statistical package for the social sciences (SPSS) 3.4.3 software. The results were expressed in percentages.

RESULTS

A total number of 5381 specimens were received from various departments (Table 1) from 4959 patients. Male and Female distribution of samples is shown in Figure 1. A total of 1485 bacterial isolates were recovered from different range of clinical specimens in both inpatients and out patients. (Table 2) Distribution of gram negative organisms among the various clinical specimens is shown in Table 3.

Table 1 Specimens collected from various departments

Specimen	Urine	Blood	Pus	Sputum	Stool	Swab	P.F	A.F	CSF	S.F	Others
Frequency	2486	1002	794	661	160	87	45	43	19	12	72
Percent	46.2	18.6	14.7	12.3	2.9	1.6	0.8	0.79	0.35	0.2	1.3

(P.F –Pleural fluid, A.F – Ascitic fluid, S.F – Synovial fluid, CSF – Cerebrospinal fluid)

Table 2 Distribution of culture positive specimens

Specimen	Urine	Pus	Blood	Sputum	A.F	CSF	Stool	P.F	S.F	Swab	Others
Frequency	658	476	131	123	10	6	29	7	3	22	20
Percent	26	60	13	18.6	23.2	31.5	18	15.5	25	25	27.7

(P.F –Pleural fluid, A.F – Ascitic fluid, S.F – Synovial fluid, CSF – Cerebrospinal fluid)

Table 3 Frequency distribution of Gram Negative organisms

Organisms	Frequency	Percentage
E.coli	483	52.6
Klebsiella sp.	196	21.6
Pseudomonas sp.	167	18
Proteus sp.	38	4
Salmonella sp.	17	2
Citrobacter	8	0.8
Moraxella	3	0.32
V.cholera	2	0.2
Acinetobacter	1	0.1
Enterobacter	1	0.1
H. influenza	1	0.1

The common bacteria encountered was E.coli 483(52%), Klebsiella sp.196(21%), Pseudomonas sp.167 (18%), Proteus sp. 38(4%), Salmonella sp. 17 (2%), Citrobacter 8 (0.8%), Moraxella 3(0.3%), Vibrio 2(0.2%), and H.influenza, Acinectobacter & Enterobacter 1(0.1%). E.coli was isolated in highest rate from urine (78%), followed by pus (9%), stool (4.5%), blood and sputum (2%). (Table 4)

The sensitivity and resistant pattern of E.coli isolates to different antimicrobials were represented in the Table 5. High level of sensitivity was seen with Imipenem(99.7%), Piperacillin+Tazobactum (97%)Meropenam (95%), Nitrofurantoin (92%), Amikacin (84%), followed by Ceftazidime(58%), Gentamicin(57%) , Aztreonam(52%), and Tobramycin(51%). Among the cephalosporins, (generation I – IV) high sensitivity rate was seen with only Ceftazidime (58%), Cefazolin (48%) where as high resistance with Ceftrioxone(64%) and Cefotaxime(63%). Very high rate of resistance was seen with Ampicillin(88%), Nalidixic acid(86%), Amoxycillin/ clavulanic acid(84%), Cotrimoxazole(74%) and Piperacillin(72%). The results also revealed that moderate resistance was observed with Ciprofloxacin(66%), Norfloxacin (62%) and Levofloxacin (51%).

Table 4 Distribution of *E.coli* in culture positive specimens

Specimen	Frequency	Percentage
Urine	379	78
Pus	45	9
Stool	22	4.5
Blood	11	2
Sputum	10	2
Ascitic fluid	3	0.6
Swab	7	1
Others	6	1

Table 5 Sensitivity and Resistant pattern of *E.coli* isolates to different antimicrobials

DRUGS	Resistant (%)	Sensitive (%)
Amikacin	32(9)	296(84)
Amoxycillin	3(60)	2(40)
Amoxycillin+clavulanic acid	11(84)	2(16)
Ampicillin	177(88)	21(12)
Azithromycin	6(46)	6(46)
Aztreonam	21(47)	23(53)
Cefazolin	12(52)	11(48)
Cefixime	14(58)	10(42)
Cefotaxime	146(63)	80(37)
Ceftazidime	106(34)	180(58)
Ceftriaxone	150(64)	81(3)
Ciprofloxacin	35(66)	18(33)
Co-trimoxazole	198(74)	68(26)
Gentamicin	102(43)	150(57)
Imipenem	1(0.3)	436(99.7)
Levofloxacin	49(51)	44(46)
Meropenem	1(4.7)	20(95)
Nalidixic acid	118(86)	18(14)
Nitrofurantoin	4(8)	60(92)
Norfloxacin	99(62)	59(37)
Penicillin	19(79)	5(20)
Piperacillin	258(72)	93(28)
Piperacillin + Tazobactum	1(3)	35(97)
Tobramycin	43(48)	51(52)

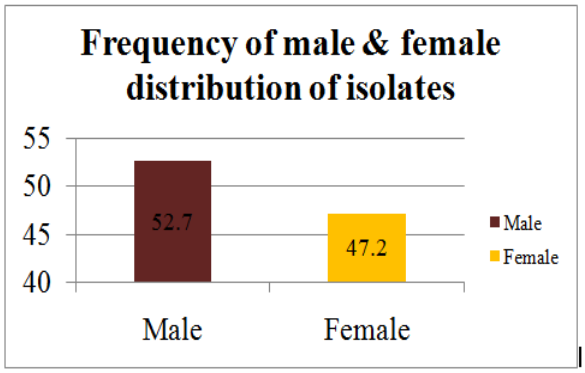

Figure 1 Frequency of male & female distribution of isolates

DISCUSSION

E.coli has been widely implicated in various clinical infections of both hospital acquired and community acquired infections. Clinicians should be aware of the raising resistance of common organisms to commonly prescribed antimicrobials. In this study, number of specimens collected from females (52.7%) was more than males (47.2%).

In our study, gram negative bacteria accounted for about 62% of the isolates. The most frequently isolated organism was E.coli (41%) which is similar to other studies (Poonam Verma 2012; Olowe et al 2008). The frequency of other organisms were Klebsiella sp.(21%), Pseudomonas sp.(18%), Proteus sp.(4%), Salmonella sp. (2%).This is supported by a study conducted in Maharashtra. (Prasad *Gunjal et al.,* 2012).Prasad Gunjal2012**.** This is supported by a study conducted in Maharashtra. Moreover E.coli was isolated in highest rate from urine (78%), pus (9%), stool (4.5%), blood and sputum (2%) (Kibret and Abera 2011).

Among the aminoglycosides tested, the maximum sensitivity was observed with Amikacin (84%) followed by Gentamicin (57%) and Tobramycin(52%). Amikacin which showed the highest susceptibility to all the isolates of E.coli in this study was reinforced by the study conducted by Mutate *et al.,* 2004 Moreover sensitivity pattern of Escherichia coli also showed higher rates to Imipenem (99.7%) followed by Piperacillin &Tazobactam (97%), Meropenam (95%) and Nitrofurantoin (92%) which was in accordance with the findings of the study done by Syed Mustaq Ahmed *et al.,*2012. Surprisingly isolates in this study were highly sensitive to Nitrofurantion (92%). **.** Extreme sensitivity of E. coli isolates to nitrofurantion has been reported in earlier study (Bonten *et al*.,1990).

Among the Cephalosporins tested (generation I – IV) high sensitivity rate was seen with Ceftazidime (58%), and Cefazolin (48%) only where as high resistance rates was seen with Cefuroxime (100%), Ceftriaxone(64%), Cefotaxime (63.7%), and Cefazolin(52%). High level of resistance to Cephalosporins suggests that resistance observed may be mainly due to production of beta-lactamases. Antimicrobial resistance, particularly to Fluoroquinolones and third-generation Cephalosporins has been increasing for E. coli (Oteo et al 2002).

The E. coli isolates of most of the specimens exhibited a high rate of resistance to Ampicillin, Amoxicillin, Amoxicillin – clavulanic acid, Co-trimoxazole, Ciprofloxacin, Nalidixic acid and Norfloxacin. Studies have shown that a high rates of resistance of E. coli to Ampicillin, Amoxicillin, Tetracycline and Trimethoprim – Sulfamethoxazole (Praseeda desai *et al.,* 2012).

CONCLUSION

Despite efforts to limit the rapid rise of antimicrobial resistance, the problem of developing resistance to multiple antimicrobials continues to worsen as shown by various studies including the present study. There is an alarmingly high rate of resistance to Cephalosporins, Fluroquinolones and Penicillins against E.coli. This clearly indicates that antimicrobial resistance to commonly used drugs is high in our region. Our current study indicated that there is a need to develop antibiotic policy and this will provide valuable insight on resistance trends and encourage the prudent use of antibiotics, which is a major factor in controlling the emergence and spread of resistant strains.

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