Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (Online)**
Sch. J. App. Med. Sci., 2014; 2(3A):927-931 **ISSN 2347-954X (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublisher.com

Research Article

Distribution and Antibiogram of Gram Negative Isolates from Various Clinical Samples at a Teaching Hospital, Tumkur

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Abstract: Infection due to Gram-negative bacteria (GNB) has become an ever increasing problem in recent years. The development of antibiotic resistance limits the choice of antibiotics to be used. Widespread irrational antibiotic usage is leading to a greater trend towards antibiotic resistance. This study was conducted to know the antibiogram of Gram negative bacilli isolated from various clinical samples in our teaching hospital**.** The present study is a retrospective study conducted in Shridevi Institute of Medical Sciences and Research hospital from November 2012 to October 2013. Gram negative isolates from all clinical samples were included in the study. Samples were processed by standard microbiological techniques. Gram negative isolates constituted 69.4% of the total culture positives. Enterobacteriacea were seen among 84.8% of the Gram negative isolates. *E.coli* was the most common bacteria isolated constituting 55.3% of the isolates.100% sensitivity was noted to imipenem, 91.1% to piperacillin /tazobactam and 87.7% to amikacin.
Keywords: Antibiogram, Gram-negative bacteria (GNB), *Enterobacteriaceae*, Nonfermenting gram negative rods.

INTRODUCTION

Infection due to Gram-negative bacteria (GNB) has become an ever increasing problem in recent years [1]. Gram-negative bacteria are common causes of intra-abdominal infections (IAIs), urinary tract infections (UTIs), nosocomial pneumonia, and bacteremia.The most common organisms responsible for these infections are multidrug resistant Gram negative bacilli particularly members of the family *Enterobacteriaceae* and nonfermenting gram negative rods.

Antimicrobial resistance among GNB is increasing worldwide. This is a major public health problem and a cause for both substantial morbidity and mortality among hospitalized patients [2].

The development of antibiotic resistance limits the choice of antibiotics to be used. Widespread irrational antibiotic usage is leading to a greater trend towards antibiotic resistance. Lack of local antibiotic policy in most of the settings is further exerting a selective antibiotic pressure selecting out resistant strains [3].

This study was conducted to know the antibiogram of Gram negative bacilli isolated from various clinical samples in our teaching hospital.

MATERIALS AND METHODS

The present study is a retrospective study conducted in Shridevi Institute of Medical Sciences and Research hospital from November 2012 to October 2013. Gram negative isolates from all clinical samples were included in the study. The clinical samples included urine, pus, blood, stool and miscellaneous samples which include fluids, vaginal swab etc.

All the clinical samples were streaked on blood agar, Macconkey agar and chocolate agar (for sputum and fluids). For blood cultures blood was inoculated into Brain heart infusion broth which was later subcultured on blood agar and macconkey agar. The plates were incubated at 37^0C for 24 to 48hrs. The growths on the plates were identified by standard microbiological techniques.

The isolates were tested for their antimicrobial susceptibility patterns by the modified Kirby-Bauer disk diffusion method on Mueller Hinton agar. Their sensitivities to ampicillin (10 µg), amoxyclav (20/10 µg), ciprofloxacin (5 µg), norfloxacin (10µg), amikacin (30µg), gentamicin (10µg), cefuroxime (30µg), cefepime (30µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), cotrimoxazole(1.25/23.75µg), imipenem (10 µg),

piperacillin/tazobactam (100/10µg) for all isolates and in addition nitrofurantoin (300µg) for urinary isolates, were tested according to the Clinical Laboratory Standard Institute guidelines. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as the control strains for the identification and the antimicrobial susceptibility tests.

RESULTS

A total of 967 samples were received in the microbiology lab of our hospital.

Table 1: Sample wise distribution

Sample	Number
Urine	659
Pus	147
Sputum	75
Blood	58
Stool	20
Miscellaneous	8
Total	967

Table 2: Table showing total number of culture positives

Sample	Total culture positives	% of gram negative bacilli
Urine	237	75.9%
Pus	112	55.3%
Sputum	31	67.74%
Blood	9	55.5%
Miscellaneous	1	100%

Table 3: Organism wise distribution in various samples

	Urine	Pus	Sputum	Blood	Misellaneous	Stool	Total
Enterobacteriaceae							
E.coli	125	21		3	1		150
Klebsiella spp	23	8	14				45
Enterobacter spp	7						7
Citrobacter spp	6	2	2				10
Proteus spp	2	10					12
Providencia spp	4	1					5
Shigella spp	0					1	1
Other Gram negative bacilli							
Pseudomonas spp	10	14	4				28
Non fermenting Gram negative bacilli	3	6	2	2			13

E. coli was the most common isolate constituting 55.3% of the total isolates, followed by *Klebsiella spp* constituting 16.6%.

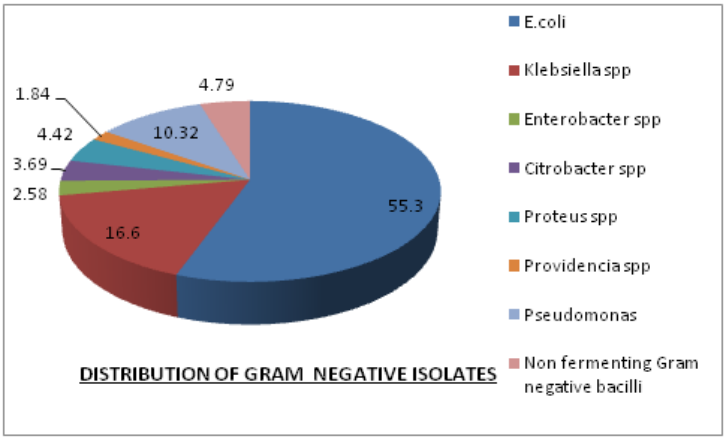

Fig. 1: Percentage of organisms isolated in various clinical samples

Table:4 Sensitivity pattern of various organisms

	E.coli (n=150)	*Klebsiella spp (n=45)*	*Enterobacter spp (n=7)*	*Citrobacter spp (n=10)*	*Proteus (n=12)*	*Providencia (n=5)*	*Pseudomonas spp (n=28)*	NFGNB *(n=13)*
A	12 (8%)			0	3(25%)	5 (100%)		0
AMC	51(34%)	2(4.44%)	2(28.5%)	3(30%)	7(58.3%)	5(100%)		2(15.3%)
CIP	91(60%)	31(68.8%)	6(85.7%)	4(40%)	12(100%)	5(100%)	10(35.7%)	10(77%)
NX	88(58.6%)	31(68.8%)	6(85.7%)	4(40%)	12(100%)	5(100%)	10(35.7%)	10(77%)
CXM	41(27.3%)	4(8.8%)	0	0	7(58.3%)	5(100%)		9(69.2%)
CRO	41(27.3%)	11(24.4%)	0	0	7(58.3%)	5(100%)		9(69.2%)
CZ	41(27.3%)	11(24.4%)	0	0	7(58.3%)	5(100%)	11(39.2%)	9(69.2%)
CEFI	44(29.3%)	15(33.3%)	0	0	7(58.3%)	5(100%)	15(53.5%)	9(69.2%)
COT	54(36%)	9(20%)	3(42.8%)	2(20%)	3(25%)	3(60%)		12(92%)
AK	142(98%)	37(82.2%)	7(100%)	6(60%)	6(50%)	5(100%)	22(78.5%)	12(92%)
GEN	105(72%)	32(71.1%)	5(71.4%)	6(60%)	8(66%)	5(100%)	19(67.85%)	12(92%)
IMP	150 (100%)	45(100%)	7(100%)	10(100%)	12(100%)	5(100%)	28(100%)	13(100%)
PIP/TAZ	146(97.3%)	33(73.3%)	7(100%)	6(60%)	12(100%)	5(100%)	26(92.8%)	11(84.6%)
NIT	116(77.3%)	18(40%)	4(57.1%)	3(30%)	0	5(100%)		3(23%)

(A- Ampicillin, AMC- amoxyclav,CIP- ciprofloxacin, NX- norfloxacin, CXM- cefuroxime, CRO- ceftriaxone,CZ- Ceftazidime, CEFI- cefipime, COT- cotrimoxazole, AK- amikacin, GEN- gentamycin, IMP- imipenem, PIP/TAZ- piperacillin/tazobactam, NIT- nitrofurantoin)

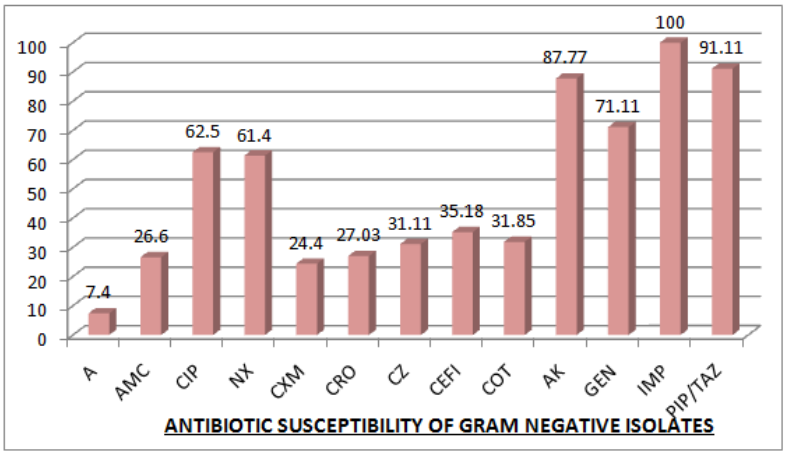

Fig. 2: Antibiotic susceptibility pattern of Gram negative isolates

DISCUSSION

The microbial pathogens, as well as their antibiotic sensitivity patterns may change from time to time and place to place. The discovery of antibiotics revolutionised the management of infectious diseases. However, the overuse and misuse of antibiotics is leading to the emergence of resistance to these life – saving drugs. Hospital antibiograms are commonly used to help guide empiric antimicrobial treatment and are an important component of detecting and monitoring trends in antimicrobial resistance [4].

Gram negative isolates were predominant isolates among all samples constituting 69.4%.

Members of the family Enterobacteriaceae are among the most important bacterial human pathogens accounting for the majority of bacteria isolated from clinical samples [5]. In our study 84.8% of the Gram negative isolates belonged to enterobacteriacea. Similar results were observed in a study by Balan K *et al*. [6].

In a study by Vipin Kumar *et al*. [7] 52 (58.42%) isolates of *Escherichia coli* were found to be the most common organisms followed by *Klebsiella pneumoniae* 18 (20.22%), *Pseudomonas aeruginosa* 11 (12.35%), *Proteus vulgaris* 3 (3.37%), *Proteus mirabilis* 2 (2.24%), *Enterobacter aerogenes* 2 (2.24%). Similar results were observed in our study.

Resistance emerges from over utilization of antibiotics trying to sterilize the environment and also the inappropriate use of the antibiotics for treatment. Free availability and self medication of antibiotics, lack of access to health facilities, in adequate public awareness, uncontrolled antibiotics use in agriculture,

lack of adequate antimicrobial resistance surveillance and lack of updated national antibiotic polices and guidelines are added worries. Antibiotics are commonly used in animals for prophylaxis or as performance enhancer and such practices are likely to increase the development of resistance [8].

Unfortunately, bacteria have developed several mechanism of resistance mechanism of resistance against various antibiotics such as synthesis of drug inactivating enzymes like β lactamases which hydrolyses the β lactam antibiotics, decreased target susceptibility by target alteration, development of efflux system and modification of diffusion barrier, altered metabolic activity [6]. Raghunath *et al*. reported from India in 2008 that coliforms have changed their susceptibility patterns extensively. According to them, β-lactam resistance is widespread among Coliform bacteria due to vertical as well as horizontally acquired resistance factors [9].

High amount of resistance was noted to ampicillin, amoxyclav, cephalosporins. Similar results were observed in a study by Iffat Javed *et al*. [10] and Balan K *et al*. [6]. In our study 70 to 75% resistance was noted to cephalosporins, similar sensitivity pattern to cephalosporins was noted in a study by Mohamaad Mehr *et al*. [11]. Hena rani *et al*. [12] showed that Extended Spectrum Beta Lactamases production was noted in 66% of the isolates.

Fluoroquinolones were sensitive in 60% of *E.coli* and 68% of *Klebsiella spp*, but only 35% sensitivity among *Pseudomaonas spp*. Similar results were observed in a study by Hossam M Ashour *et al* in which *Klebsiella spp* showed 60% sensitivity and *Pseudomonas spp* 45% sensitivity however *E.coli* isolates in their study showed only 33% sensitivity [13].

High sensitivity was noted to amikacin and gentamycin among enterobacteriacea in our study. Similar results were observed in a study by Krithu Panta *et al*. [8].

For Cotrimoxzole around 30% sensitivity was seen among Gram negative isolates similar results were noted in a study by Hung Ming Cheng *et al*. [14].

In our study maximum sensitivity was seen for imipenem (100%), piperacillin/tazobactam (91.1%) and amikacin(87.7%). Similar results were observed by Balan K *et al*. [6] which showed high susceptibility to Imipenem, amikacin and piperacillin/tazobactam.

The antimicrobial agents are losing their efficacy because of the spread of resistant organism due to indiscriminate use of antibiotics, lack of awareness, patient non compliance and unhygienic condition.

CONCLUSION

Gram negative isolates are the most common cause of infections. High amount of antibiotic resistance is noted among the isolates. Hence to prevent the spread of the resistant bacteria, it is necessary to have antibiogram for hospitals to know the common organisms and their susceptibility patterns.

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