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Distribution Pattern of Gram-Negative Bacteria in Various Clinical Isolates and Is Antibiotic Sensitivity Pattern in A Tertiary Care Teaching Hospital

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Abstract

INTRODUCTION

Antimicrobial resistance is really a threat to clinical practice. Bacteria develop various mechanisms of resistance to different group of antibiotics. Antimicrobial resistance is the resistance of a microorganism to an antimicrobial drug that was originally effective for treatments of infections caused by it, by acquiring genes coding for it the consequences of antibiotic resistance led to longer duration of illness, treatment with expensive drugs, higher mortality and increased burden on the health system. The present study aims to find out the various clinical isolates having gram negative bacteria and its antibiotic sensitivity pattern.

MATERIALS AND METHODS:

A prospective study was conducted from April 2018 to March 2019 in the department of Microbiology, Isolates obtained from various clinical samples sent to the microbiology laboratory were identified by standard microbiological techniques. The antimicrobial susceptibility testing was carried out by the disc diffusion method by Kirby Bauer according to CLSI guidelines.

RESULTS:

In our study ,204 clinical isolates were collected, Urine was the commonest clinical isolate and E.Coli was the commonest clinical organism seen in our study followed by klebsiella. Coming to antibiotic sensitivity it was highest for Imipenam (87.8%) followed by Amikacin (83.1%). Similar sensitivity pattern was also seen in individual species too.

CONCLUSION:

The study conducted in our hospital highlights the emerging prevalence of beta lactamases. The consequences of antibiotic resistance led to longer duration of illness, treatment with expensive drugs, higher mortality and increased burden on the health system. We must have a functional hospital infection control committee with appropriate antibiotic policy and regular updates, to prevent resistance.

Keywords: Gram negative, E.Coli, Antibiotic sensitivity.

INTRODUCTION

Antimicrobial resistance is really a threat to clinical practice. Bacteria develop various mechanisms of resistance to different group of antibiotics. Antimicrobial resistance is the resistance of a microorganism to an antimicrobial drug that was originally effective for treatments of infections caused by it, by acquiring genes coding for it. Antimicrobial resistance is a global concern, as new resistance mechanism evolves, making the treatment of even common infections difficult resulting in disability or death of individual.

In the early 1980s, the third-generation Cephalosporins which were very effective were introduced for treating beta lactamase mediated resistance. The advantages of these drugs were decreased nephrotoxic effects when compared to Aminoglycosides and Polymyxin. 1 E.coli and Klebsiella are leading causes of pneumonia, UTI, diarrhea, cholecystitis, meningitis. β lactam antibiotics has been the drug of choice in treating these conditions. However multiple antibacterial resistances in recent decades among Enterobacteriaceae are commonly met and the above conditions and pose a problem in management. 2

Over the past few years, the occurrence of multidrug-resistant Gram- negative bacteria has increased due to multiple mechanism of resistance, which is a continuous phenomenon. The major concern is the production of AmpC beta-lactamases and/or extended-spectrum beta-lactamase in bacterial strains. ESBL's confer resistance to, Cephalosporins, Oxyimino-cephalosporins (e.g., Ceftriaxone, Cefotaxime, and ceftazidime) Amino- penicillins and AmpC beta-lactamases in addition are resistant to Cephamycin (e.g., Cefoxitin) and Monobactams. 3

A prospective study was conducted from April 2018 to March 2019 in the department of Microbiology, Chengalpet Medical College, Chengalpet district. The present study aims to find out the antibiotic susceptibility pattern of gram-negative bacteria from the samples received from outpatients and inpatients, who were admitted to different wards in our hospital.

METHODOLOGY

Isolates obtained from various clinical samples sent to the microbiology laboratory were identified by standard microbiological techniques. The antimicrobial susceptibility testing was carried out by the disc diffusion method by Kirby Bauer according to CLSI guidelines. Antimicrobial discs [Himedia Mumbai] used were: Amikacin (30ug), Ampicillin (10 µg), Amoxicillin- clavulanic acid (30/10µg), Gentamicin (10ug), Ciprofloxacin (5µg), Cotrimoxazole (1.25/23.75µg) Ceftriaxone (30 µg), Cefotaxime (30µg), Ceftazidime (30µg) and Imipenem (10µg). Quality control was achieved by using E.coli ATCC 25922. The isolates showing resistance to any of the 3GC were stored in stock vials for further processing. Media and disks were tested for quality control with standard strains.

All gram-negative clinical isolates from both sexes of all age group were included in the study. Non Enterobacteriaceae isolates were excluded. Those samples which showed mixed growth were excluded. Isolates sensitive to all antimicrobial disc tested were exclude. The isolates stored in the stock vials were sub cultured at the time of testing. The purity and viability of the isolates was checked. The observations of the study were recorded and analyzed. The results were compared and discussed by using SSPS software and Chi Square test.

RESULTS

In our study 204-gram negative samples were obtained during the study period among which when we analyzed age wise distribution of isolates it was falling in the age group of 40 -60 years (44.12%) followed by 20-40 years (29.4%) and more than 60 years (18.1%) and rest below 20 years of age. Next, we analyzed the sex wise distribution of isolates. The clinical isolates were obtained from 97 male patients and 107 female patients.

Among 204 resistant clinical isolates, 174 isolates were from inpatients and 30 were from outpatients. The various clinical specimens from which these isolates were obtained are as follows- urine (100), pus (58), sputum (30), wound swab (4), cervical swab (2), high vaginal swab (2), aural swab (1), catheter tip (3), tracheal tip (1), tissue (2) and blood (1). The results are shown below.

Table 1: Distribution of isolates in samples

Specimens	No. of samples(n=204)	Percentage %
Urine	100	49.01
Pus	58	28.43
Sputum	30	14.71

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Wound swab	4	1.96
Cervical swab	2	0. 98
Catheter tip	3	1.47
Tissue	2	0.98
High vaginal swab	2	0.98
Aural swab	1	0.49
Blood	1	0.49
Tracheal tip	1	0.49
Total	204	100

In our study the urinary tract infections were predominant 49.01%, (100/204), followed by skin and soft tissue infections 30.4% (pus samples 58/204 and wound swab 4/204) and respiratory infections was 14.71%. (30/204).

Among the 204 samples 174 samples were from inpatients, in which 44 were from ICU and 142 were from various wards. The number of isolates from each ward was as follows:19 from male medical ward, 16 from female medical ward 38 from male surgical ward ,18 from female surgical ward 6 from pediatric ward, 32 from Gynecology ward, 1 from ENT ward and 30 from OPD.

Among the 204 isolates, 161 isolates belong to family Enterobacteriaceae and non-fermenters constituted 43 isolates. Out of the 161, 105 samples showed growth of E.coli, 51 had Klebsiella species, 02 were Enterobacter species and 03 were Citrobacter species. Among the non-fermenters, 37 were Pseudomonas species and 06 belong to Acinetobacter species.

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Table 2: Number of GNB isolated

Organism	**Number**	**Percent**
E.coli	105	51.47%
Klebsiella spp.	51	25%
Pseudomonas spp.	37	18.14%
Acinetobacter spp.	6	2.94%
Citrobacter spp.	3	1.47%
Enterobacter spp.	2	0.98%
Total	204	100%

E.coli was the common organism isolated among the samples from various wards. In the medical ward the common organism isolated was E. coli (54.3%) followed by Pseudomonas spp. (25.7%). It was observed that E .coli was also common organism among the specimens of surgical wards (38.6%) and ICU (48.9%).

Klebsiella species was the second common isolate in ICU specimens (33.3%) and in surgical wards (29.8%). Pseudomonas spp. was almost equal among the specimens from various wards in IPD except in Pediatric ward which showed the least number of isolates.

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Coming to organism isolated from specimens E.coli was most common organism isolated from urinary samples 76/105 (76%), followed by Klebsiella spp. 14/100 (14%). In pus samples E.coli constituted (36.2%) and Klebsiella spp. 34.5%. In sputum samples Klebsiella spp. and Pseudomonas spp. were almost equal, 40% and 46% respectively.

The antibiotic susceptibility test was done by Kirby Bauer technique to determine the susceptibility pattern of isolates which are shown below in Table 8. The interpretation was done according to CLSI guidelines 2014.

Table 3: Antibiotic susceptibility pattern

Antibiotics dose (µg)	Clinical isolates n=204	Sensitivity Percentage
Ampicillin (10)	5	2.45%
Amoxy clav(20/10)	13	6.37%
Cotrimaxole(1.25/23.75)	43	21.10%
Ciprofloxacin(5)	66	32.40%
Nitrofurantoin(10)	82	40.20%
Gentamicin (10)	91	44.62%
Amikacin(30)	170	83.31%
Imipenem(10)	179	87.80%

In our study sensitivity to Quinolones, Cotrimaxole, Nitrofurantoin and Gentamicin varied from 20% - 44%. Most of the species were highly sensitive to Amikacin and Imipenem, 83.3% and 87.8% respectively.

Antibiotic susceptibility test was done by disc diffusion method as per CLSI guidelines. The susceptibility of various isolates to the drugs is shown below.

Table 4 : Antibiotic susceptibility pattern of various isolates

Name of GNB	Ampi	Cipro	Genta	Amikac	Nitro	Cotri	Ceftazi	Cefotax	Ceftri	Imp	Amc
E.coli	4 (3.8%)	22 (20.9%)	35 (33.3%)	93 (88.6%)	65 (61.9%)	20 (19%)	6 (5.7%)	10 (9.5%)	14 (13.3%)	94 ((89.5%)	10 (9.5%)
Klebsiella	0	17 (33.3%)	24 (47%)	38 (74.5%)	13 (25.5%)	17 (33.3%)	6 (11.8%)	5 (9.8%)	10 (19.6%)	43 (84.3%)	2 (3.9%)
Pseudomonas	0	25 (67.6%)	27 (72.9%)	34 (91.9%)	2 (5.4%)	3 (8.1%)	13 (35.1%)	11 (29.7%)	12 (32.4%)	30 (81%)	0 (%)

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Acinetobacter	0	2 (33.3%)	3 (50%)	4 (66.7%)	0 (%)	2 (33.3%)	0	1 (16.7%)	0	3 (50%)	0 (%)
Enterobacter	0	0	0	0	0	0	0	0	0	2 (%)	0 (%)
Citrobacter	1 (33.3%)	0 (%)	2 (66.7%)	1 (33.3%)	2 (66.7%)	1 (33.3%)	0	0	0	3 (100%)	1 (33.3%)

Most of the species were resistant to Ampicillin. Among the E.coli (n =105) resistance to Ciprofloxacin was observed in 83 cases , Cotrimaxole resistance in 85 cases , Nitrofurantoin resistance in 40 cases, and resistance to and Amikacin was 11 and Imipenem was 12 cases..

Of the 51 isolates of Klebsiella species included in the study resistance to Ciprofloxacin was observed in 34 cases, Cotrimaxole resistance in 34 cases, Nitrofurantoin resistance in 48 cases, and resistance to Imipenem and Amikacin was observed in 08 and 13 cases respectively.

Of the 37 isolates of Pseudomonas spp., 12 were resistance to Ciprofloxacin, 34 were Cotrimaxole resistance, resistance to Amikacin and Imipenem was 13 and 07 respectively.

DISCUSSION

Antimicrobial resistance is a growing threat worldwide. Increasing resistance to third generation cephalosporins has become a cause for concern among Enterobacteriaceae.4 The efficacy of beta lactam antibiotics has been reduced by production various types of beta lactamases. The members of Enterobacteriaceae are the most frequent human pathogen in causing infections and isolated many a times from clinical samples.

In the present study, a total of 204 isolates showing resistance to Ceftazidime, Cefotaxime or Ceftriaxone were selected from 492 GNB samples. These consisted of urine constituting 49.01% (100/204), pus 28.43% ((58/204) and sputum 14.7% (30/204) .

Our study showed that among the isolates, E. Coli was predominant accounting to 51.47% (105/204) followed by Klebsiella spp about 25% (51/204), Pseudomonas spp. 18.13% (37/204) Acinetobacter spp 2.94% (6/204) Citrobacter spp. 1.47% (3/204) and Enterobacter spp. 0.98% (2/204). This finding was on par with other studies by Metri et al5 and Mathur et al, where the E.coli was the predominant organism isolated.

The antibiotic sensitivity pattern of beta lactamase producers revealed that maximum susceptibility was seen for Imipenem and Amikacin 87.8%, and 83.3% respectively. Sensitivity to Quinolones, Cotrimaxole, Gentamicin and Nitrofurantoin was 32.4%, 21.1%, 44.62%, and 40.2% respectively.

Most of the isolates showed decreased susceptibility to Ampicillin and Amoxy clav drugs, 2.45% and 6.37% respectively.

Most isolates were susceptible to Imipenem except 25 isolates (25/204). Resistance to Meropenem was also detected and it was found in 28 isolates (28/204). (Table13) Except 3, all isolates which showed resistance to Meropenem were also resistant to Imipenem. This was confirmed by MIC, where 28 isolates showed MIC >2µg. (Table: 15)In the current study, we majority of the isolates were sensitive to Imipenem, which was concordant with other studies. Similar findings were seen in another study from Coimbatore.7

The antibiotic susceptibility profile of our study revealed that susceptibility of beta lactamase producers to Imipenem and Amikacin were found to be 87.8%, and 83.3% respectively. Sensitivity to Quinolones, Cotrimaxole, Gentamicin and Nitrofurantoin were 32.4%, 21.1%, 44.62%, and 40.2% respectively. Majority of the isolates found to have decreased susceptibility to Ampicillin and Amoxy clavulanic acid. (2.45% and 6.37%)

CONCLUSION

The study conducted in our hospital highlights the emerging prevalence of beta lactamases. The consequences of antibiotic resistance led to longer duration of illness, treatment with expensive drugs,

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higher mortality and increased burden on the health system. We must have a functional hospital infection control committee with appropriate antibiotic policy and regular updates, to prevent resistance. Each and every antibiotic usage in hospital should be documented which will help us to develop dosing strategies

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