

Surveillance of Antimicrobial Resistance among Gram Negative Bacteria over a One Year Period at a Tertiary Care Hospital

Dr. B. Hemanath¹, Dr. K. Madhurima^{2*}, Dr. G. Jyothi Lakshmi³, Dr. P. Shashikala Reddy⁴¹Post graduate, Osmania Medical College, Hyderabad, Telangana, India²Associate Professor, Osmania Medical College, Hyderabad, Telangana, India³Professor, Osmania Medical College, Hyderabad, Telangana, India⁴Professor & HOD, Osmania Medical College, Hyderabad, Telangana, India

*Corresponding author: Dr. K. Madhurima

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Abstract

Original Research Article

Background: Sepsis, skin & soft tissue infections, intra-abdominal infections, urinary tract infections and respiratory tract infections caused by Gram negative bacteria are most common in clinical laboratories. The acquired resistance of most bacteria to antimicrobials is unpredictable and requires routine antimicrobial susceptibility testing. **Aims & Methods:** This study was conducted during one-year period to determine antimicrobial resistance among Gram-negative bacilli in common clinical infections and to find antimicrobial sensitivity pattern of resistant isolates. Samples were cultured on Mac Conkey agar and Blood agar and identified by standard microbiological techniques. Antimicrobial susceptibility testing was done by Kirby-Bauer disk diffusion method and resistance patterns were recorded. Antimicrobials tested were Cephalosporins (Ceftazidime), β -lactam/ β -lactamase inhibitor (Cefoperazone, Piperacillin, Cefoperazone/Sulbactam & Piperacillin/Tazobactam), carbapenems (Imipenem & Meropenem), monobactam (Aztreonam), fluoroquinolones (Ciprofloxacin & Ofloxacin), Cotrimoxazole and aminoglycosides (Amikacin). Screening of ESBL producers and Carbapenemase producers was done by using Ceftazidime and Imipenem disks as markers respectively. Quality control was performed using ATCC strains every week. **Results:** Among the total 2399 Gram-negative bacterial pathogens isolated, *Klebsiella spp* were 1156 (48.2%), *E. coli* were 844 (35.2%), *Pseudomonas spp* were 276 (11.5%) & others were 123 (5.1%). Most GNB were sensitive to carbapenems followed by Cefoperazone/Sulbactam & Piperacillin/Tazobactam. ESBL positive rates ranged from 77% – 80% in *E. coli*, 75% - 78% in *Klebsiella spp* & 70%– 74% in *Pseudomonas spp*. Carbapenem resistance rates were 3-7% & was high among *Pseudomonas spp*. **Conclusion:** There is an increasing trend of ESBL production among common Gram-negative bacterial pathogens. Carbapenems & Cefoperazone/Sulbactam are better agents though resistance to carbapenems is slowly increasing especially among *Pseudomonas spp*. Continuous monitoring & AMR detection may guide in therapy of resistant cases. Antimicrobial surveillance studies help to formulate antibiotic policy which is a tool for clinicians to decide on empirical therapy preventing overuse of antibiotics.

Key words: Antimicrobial Resistance, Carbapenemase producer, ESBL producer, Intra-abdominal infections, LRTI, Sepsis, Surveillance, UTI.

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INTRODUCTION

Sepsis, skin & soft tissue infections, intra-abdominal infections, urinary tract infections and respiratory tract infections caused by Gram negative bacteria are most common entities in clinical laboratories. The acquired resistance of most bacteria to antimicrobials is unpredictable so it requires routine antimicrobial susceptibility testing [1]. The resistant profiles vary significantly in different locations world-wide [2]. Hence it is important to do surveillance in antimicrobial susceptibility of common pathogens. This helps to monitor changing trend in antibiotic resistance over time to guide proper therapeutic strategies & to

overcome infections by drug-resistant pathogens [2]. There is rise in Antimicrobial resistance [AMR] world-wide especially in Asian countries as evident from previous studies [3]. It is due to beta lactamases, in particular extended spectrum beta lactamases and carbapenemases which are spreading rapidly due to mobile genetic elements [3]. The aim of this study was to look for the antibiotic resistance in Gram negative bacteria in different clinical conditions and to find antimicrobial sensitivity pattern of resistant isolates.

MATERIALS AND METHODS

This study included a total of 2399 Gram negative bacteria from sepsis, skin & soft tissue

infections (SSTI), intra-abdominal infections (IAI), urinary tract infections (UTI) and respiratory tract infections (RTI) at a tertiary care hospital, Hyderabad during December 2017 to November 2018. Samples were initially subjected to Gram's stain. Then cultured on Mac Conkey agar and Blood agar, incubated for 24–48 hrs. at 37°C. Colonies were identified by standard microbiological techniques & antimicrobial susceptibility testing was done by Kirby Bauer disk diffusion method matching 0.5 McFarland Standards. Isolates were identified up to species level. Susceptibility pattern were recorded. Antimicrobials tested were Cephalosporins (Ceftazidime), β -lactam/ β -lactamase inhibitor (Cefoperazone, Piperacillin, Cefoperazone/Sulbactam & Piperacillin/Tazobactam),

carbapenems (Imipenem & Meropenem), monobactam (Aztreonam), fluoroquinolones (Ciprofloxacin & Ofloxacin), Cotrimoxazole and aminoglycosides (Amikacin). Carbapenem susceptible isolates were screened for ESBL production using Ceftazidime disk as marker drug. Screening of carbapenemase production was done using Imipenem disk as marker drug. Quality control was performed using *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 and *Enterococcus faecalis* ATCC 29212 every week. Every batch of media was tested for sterility and performance.

RESULTS

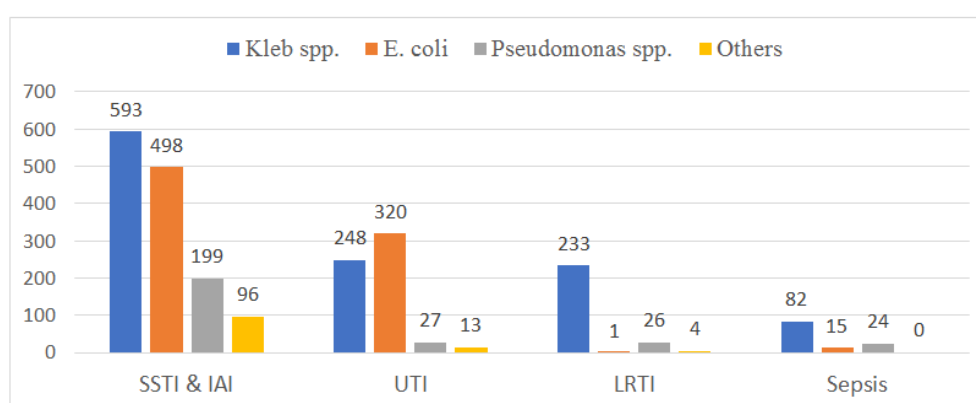


Fig-1: Distribution of isolates among common clinical conditions

Among all samples during one year, a total of 2399 Gram negative bacterial isolates were obtained. *Klebsiella* spp was most common (1156; 48.2%) followed by *Escherichia coli* 844 (35.2%), *Pseudomonas* spp 276 (11.5%) and others 123 (5.1%) like *Acinetobacter baumannii*, *Proteus* spp, *Citrobacter* spp & *Enterobacter* spp. The ESBL rates of *E. coli* was highest (77-80%) followed by *Klebsiella* spp (75-78%) and *Pseudomonas* spp (70-74%) and carbapenem resistance rates were 5–7% for *Pseudomonas* spp, 4 – 6% for *Klebsiella* spp 3 – 5% for *E. coli* as in Table I. In sepsis, IAI, skin & soft tissue infections and LRTI, *Klebsiella* spp was common followed by *E. coli* and *Pseudomonas* spp whereas in UTI, *E. coli* was most common followed by *Klebsiella* spp as shown in Fig 1. The antimicrobial susceptibility profiles of Gram-negative bacteria showed higher sensitivity to Imipenem & Cefoperazone/Sulbactam followed by Piperacillin/Tazobactam as in table II. Antimicrobial

susceptibility in general is less for *Klebsiella* spp compared to *E. coli* & *Pseudomonas*.

DISCUSSION

In Asia, the prevalence of ESBLs in intra-abdominal infections increased over time followed by Europe, Latin America, Middle East, North America and South Pacific. Conversely the trend of ESBLs in IAI decreased over a time in Africa. In UTI changing prevalence of ESBLs is less severe with only significant increases found in the Middle East and Asia [3]. Antimicrobial surveillance is need of the hour because rising antimicrobial resistance in recent days is leading to increased morbidity and mortality. WHO has introduced awareness programs to overcome this antimicrobial resistance? GLASS (Global Antimicrobial Surveillance System) was introduced in 2015 by WHO to monitor the antimicrobial resistance trend. As of May 2018, countries enrolled for GLASS are 58 including India [4].

Table-I: Distribution of Gram-negative pathogens with resistance trend

Gram negative pathogen	n	ESBL rate	Carbapenem resistance
<i>Klebsiella</i> spp.	1156(48.2%)	75 – 78%	4 – 6%
<i>E. coli</i>	844(35.2%)	77 – 80%	3 – 5%
<i>Pseudomonas</i> spp.	276(11.5%)	70 – 74%	5– 7%
Others	123(5.1%)		
Total	2399		

Table-II: Antimicrobial susceptibility profile of Gram-negative bacteria

Clinical condition	COT	OF	CAZ	AT	PTZ	CFS	IPM
SSTI & IAI							
i. <i>Klebsiella spp</i>	20.9%	49.7%	20%	43.1%	62.8%	92.5%	95.2%
ii. <i>E. coli</i>	30%	45.8%	16.7%	40.8%	74.2%	94.0%	94.8%
iii. <i>Pseudomonas spp</i>	IR	60.4%	26.4%	73.2%	83.1%	86.0%	84.4%
UTI						NIT	
i. <i>Klebsiella spp</i>	-	47.3%	35.1%	78%	85.8%	72.8%	96.1%
ii. <i>E. coli</i>	-	41.7%	34.5%	70%	89.0%	86.2%	97.0%
LRTI							
i. <i>Klebsiella spp</i>	47%	65.4%	38.1%	57.1%	87.3%	92.3%	97.5%
Sepsis							
i. <i>Klebsiella spp</i>	46.1%	66.6%	25%	94%	84.3%	92.3%	100%

COT: Cotrimoxazole, OF: Ofloxacin, CAZ: Ceftazidime, AT: Aztreonam, PTZ: Piperacillin/tazobactam, IPM: Imipenem, NIT: Nitrofurantoin

Majority of the common infections are caused by Gram negative bacilli like *E. coli*, *Klebsiella spp*, *Pseudomonas aeruginosa*. In present study *Klebsiella spp* (48 %) was most common followed by *E. coli* (35.2%), *Pseudomonas spp* and others like *Acinetobacter*, *Proteus spp*, *Enterobacter* etc. In previous studies like Hawser SP *et al.* [5], Chaudhuri BN *et al.* [6] and Veeraraghavan B *et al.* 2018 *E. coli* was most common followed by *Klebsiella spp* & *Pseudomonas spp*. ESBL positive rates are higher among most common Gram-negative pathogens like *Klebsiella spp* and *E. coli*. ESBL positive *Klebsiella spp* (75 –78%) correlated with ESBL positive rates of Veeraraghavan B *et al.* study 61 –72%. ESBL positive *E. coli* (77 – 80%) correlated with Chaudhuri BN *et al.* and Veeraraghavan B *et al.* studies as in table III.

In China, ESBL producers among *Klebsiella spp* were 40.4% (2012) to 26.6% (2014) [7] and in

Spain 16.3% (2011 – 2015) [8]. For the past few years, ESBL producers among *Klebsiella spp* in India were 61-72% (2015), 55% (2008), 63% (2007) and 90.13% (2012) [11]. In India *E. coli* ESBL producers were 66%–77% (2015), 67% (2008) and 80% (2007). In China were 67.5% (2012) to 58.9% (2014) and in Spain 9.5% (2011–2015).

When susceptibility profiles of ESBL and non ESBL compared as in as in table IV and table V; Imipenem, Cefoperazone/ Sulbactam & Piperacillin/ Tazobactam had good sensitivity even among ESBL producers in *Klebsiella spp* and *E. coli*. Among carbapenem resistant *Klebsiella spp* & *E. coli*, Colistin and Tigecycline have better sensitivity. But there are some isolates which showed resistance to Colistin which emphasizes the Colistin resistance reported in recent times in other places.

Table-III: Comparison of total isolates and resistance pattern in different studies

Author(study)	Total Isolates	ESBL positive <i>Klebsiella spp</i>	ESBL positive <i>E. coli</i>	Carbapenem Resistance
Chaudhuri BN <i>et al.</i> 2011[6]	60% <i>E. coli</i> 19% <i>Klebsiella spp</i>	63%	80%	<i>Klebsiella spp</i> (6-20%) <i>E. coli</i> (4-6%)
Hawser SP <i>et al.</i> 2010[5]	62.7% <i>E. coli</i> 16.7% <i>Klebsiella spp</i>	55%	67%	8%
Fouzia B <i>et al.</i> [11] (2012 – 2013)	-	90.13%	-	-
Veeraraghavan B <i>et al.</i> [2](2015 – 16)	44% <i>E. coli</i> 25% <i>Klebsiella spp</i>	61%–72%	66%–77%	<i>Klebsiella spp</i> (39%) <i>E. coli</i> (12%)
Zhang <i>et al.</i> CHINA [7](2012–14)	45.4% <i>E. coli</i> 20.1% <i>Klebsiella spp</i>	40.4%(2012) to 26.6%(2014)	67.5%(2012) to 58.9%(2014)	<i>Klebsiella spp</i> (12 -20%) <i>E. coli</i> (2 - 4%)
R. Canton, <i>et al.</i> SPAIN [8] (2011 – 15)	56.1% <i>E. coli</i> 10.8% <i>Klebsiella spp</i>	16.3%	9.5%	<i>Klebsiella spp</i> (5%) <i>E. coli</i> (2%)
Present study 2018	35.2% <i>E. coli</i> 48% <i>Klebsiella spp</i>	75 – 78%	77 – 80%	<i>Klebsiella spp</i> (4 – 6%) <i>E. coli</i> (3 – 5%)

Table-IV: Antimicrobial susceptibility profile of ESBL vs Non ESBL & Carbapenem resistance vs susceptible of Klebsiella spp (n=1156)

Drug	ESBL(n=867)	Non ESBL(n=289)	Carbapenem Resistance(n=80)	Carbapenem Susceptible(n=1076)
Ofloxacin	30%	67%	45%	60%
Cotrimoxazole	38%	44%	7%	38%
Aztreonam	20%	65%	17%	20%
Ceftazidime	0	100%	10%	40%
Cefoperazone	0	95%	11%	37%
Cefoperazone /sulbactam	86%	92%	53%	86%
Piperacillin/tazobactam	76%	90%	30%	76%
Imipenem	100%	100%	0	100%
Colistin	-	-	60%	-
Tigecycline	-	-	62%	-

Table-V: Antimicrobial susceptibility profile of ESBL vs Non ESBL & Carbapenem resistance vs susceptible of E. coli (n=844)

Drug	ESBL(n=650)	Non ESBL(n=194)	Carbapenem Resistance(n=74)	Carbapenem susceptible(n=770)
Ofloxacin	34%	70%	39%	50%
Cotrimoxazole	30%	50%	7%	43%
Aztreonam	33%	68%	19%	19%
Ceftazidime	0	100%	11%	34%
Cefoperazone	0	96%	14%	36%
Cefoperazone /sulbactam	89%	94%	61%	76%
Piperacillin/tazobactam	80%	90%	32%	79%
Imipenem	100%	100%	0	100%
Colistin	-	-	66%	-
Tigecycline	-	-	64%	-

CONCLUSION

Among common Gram-negative bacterial pathogens, increasing trend of ESBL production was seen. Carbapenems & Cefoperazone/sulbactam show promise as of now. Though resistance to carbapenems is slowly increasing especially among *Pseudomonas spp*, they still serve as good agents. Continuous monitoring and detection of AMR may guide in therapy of resistant cases. Antimicrobial surveillance studies help to formulate antibiotic policy which is a tool for clinicians to decide on empirical therapy preventing overuse of antibiotics.

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