Scholars Journal of Applied Medical Sciences
OPEN ACCESS
Abbreviated Key Title: Sch J App Med Sci
ISSN 2347-954X (Print) | ISSN 2320-6691 (Online)
Journal homepage: www.saspublishers.com

Microbiology

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

Dr. B. Hemanath[1], Dr. K. Madhurima[2*], Dr. G. Jyothi Lakshmi[3], Dr. P. Shashikala Reddy[4]

[1]Post graduate, Osmania Medical College, Hyderabad, Telangana, India
[2]Associate Professor, Osmania Medical College, Hyderabad, Telangana, India
[3]Professor, Osmania Medical College, Hyderabad, Telangana, India
[4]Professor & HOD, Osmania Medical College, Hyderabad, Telangana, India

***Corresponding author:** Dr. K. Madhurima
| **Received:** 01.04.2019 | **Accepted:** 05.04.2019 | **Published:** 30.04.2019
DOI: 10.36347/sjams.2019.v07i04.028

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.

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^{o}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

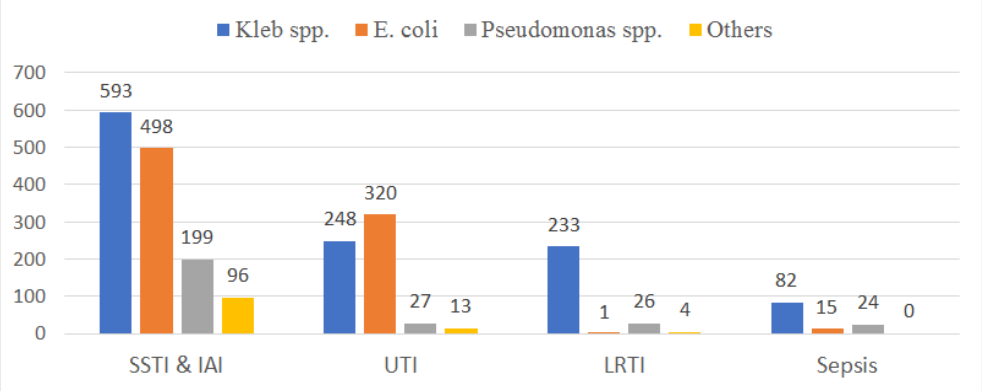

Fig-1: Distribution of isolates among common clinical conditions

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 baumanii*, *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 were5–7% 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
Klebsiella spp.	1156(48.2%)	75 – 78%	4 – 6%
E. coli	844(35.2%)	77 – 80%	3 – 5%
Pseudomonas 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. Klebsiella spp	20.9%	49.7%	20%	43.1%	62.8%	92.5%	95.2%
ii. E. coli	30%	45.8%	16.7%	40.8%	74.2%	94.0%	94.8%
iii. Pseudomonasspp	IR	60.4%	26.4%	73.2%	83.1%	86.0%	84.4%
UTI						NIT	
i. Klebsiella spp	-	47.3%	35.1%	78%	85.8%	72.8%	96.1%
ii. E. coli	-	41.7%	34.5%	70%	89.0%	86.2%	97.0%
LRTI							
i. Klebsiella spp	47%	65.4%	38.1%	57.1%	87.3%	92.3%	97.5%
Sepsis							
i. Klebsiella spp	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 Klebsiella spp	ESBL positive E. coli	Carbapenem Resistance
Chaudhuri BN *et al.* 2011[6]	60% E. coli 19%Klebsiella spp	63%	80%	Klebsiellaspp (6-20%) E. coli (4-6%)
Hawser SP *et al*. 2010[5]	62.7% E. coli 16.7%Klebsiella spp	55%	67%	8%
Fouzia B *et al.* [11] (2012 – 2013)	-	90.13%	-	-
Veeraraghavan B *et al.* [2](2015 – 16)	44%E.coli 25%Klebsiella spp	61%–72%	66%–77%	Klebsiella spp (39%) E. coli (12%)
Zhang *et al*. CHINA [7](2012–14)	45.4% E.coli 20.1%Klebsiella spp	40.4%(2012) to 26.6%(2014)	67.5%(2012) to 58.9%(2014)	Klebsiella spp (12 -20%) E. coli (2 - 4%)
R. Canton, *et al*. SPAIN [8] (2011 – 15)	56.1% E.coli 10.8%Klebsiella spp	16.3%	9.5%	Klebsiella spp (5%) E. coli (2%)
Present study 2018	35.2%E.coli 48%Klebsiella spp	75 – 78%	77 – 80%	Klebsiella spp (4 – 6%) E. coli (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.

REFERENCES

1. Tille MP. Bailey & Scott's Diagnostic Microbiology. Elsevier Publishers. 2017. 14th edition.
2. Veeraraghavan B, Jesudason MR, Prakasah JA, Anandan S, Sahni RD, Pragasam AK, Bakthavatchalam YD, Selvakumar RJ, Dhole TN, Rodrigues C, Roy I. Antimicrobial susceptibility profiles of gram-negative bacteria causing infections collected across India during 2014–2016: Study for monitoring antimicrobial resistance trend report. Indian journal of medical microbiology. 2018 Jan 1;36(1):32.
3. Morrissey I, Hackel M, Badal R, Bouchillon S, Hawser S, Biedenbach D. A review of ten years of the Study for Monitoring Antimicrobial Resistance Trends (SMART) from 2002 to 2011. Pharmaceuticals. 2013 Nov 1;6(11):1335-46.
4. https://www.who.int/glass/
5. Hawser SP, Badal RE, Bouchillon SK, Hoban DJ, SMART India Working Group. Antibiotic susceptibility of intra-abdominal infection isolates from Indian hospitals during 2008. Journal of medical microbiology. 2010 Sep 1;59(9):1050-4.
6. Chaudhuri BN, Rodrigues C, Balaji V, Iyer R, Sekar U, Wattal C, Chitnis DS, Dhole TN, Joshi S. Incidence of ESBL producers amongst Gram-negative bacilli isolated from intra-abdominal infections across India (based on SMART study, 2007 data). JAPI. 2011 May;59(16):1-6.
7. Zhang H, Yang Q, Liao K, Ni Y, Yu Y, Hu B, Sun Z, Huang W, Wang Y, Wu A, Feng X. Update of incidence and antimicrobial susceptibility trends of Escherichia coli and Klebsiella pneumoniae isolates from Chinese intra-abdominal infection patients. BMC infectious diseases. 2017 Dec;17(1):776.
8. CantónR,Loza E, Aznar J, Barrón-Adúriz R, Calvo J, Castillo F.J, Cercenado E, Cisterna R, González-Romo F, López-Hontangas JL, Suárez-

BarrenecheaAI,Tubau F, Molloy B, López-Mendoza D and SMART-Spain Working Group. Antimicrobial susceptibility trends and evolution of isolates with extended spectrum β-lactamases among Gram-negative organisms recovered during the SMART study in Spain (2011-2015). Rev EspQuimioter. 2018;31(2): 136-145.

9. Yang Q, Zhang H, Wang Y, Xu Y, Chen M, Badal RE, Wang H, Ni Y, Yu Y, Hu B, Sun Z. A 10 year surveillance for antimicrobial susceptibility of Escherichia coli and Klebsiella pneumoniae in community-and hospital-associated intra-abdominal infections in China. Journal of medical microbiology. 2013 Sep 1;62(9):1343-9.
10. Pragasam AK, Veeraraghavan B, Nalini E, Anandan S, Kaye KS. An update on antimicrobial resistance and the role of newer antimicrobial agents for Pseudomonas aeruginosa. Indian journal of medical microbiology. 2018 Jul 1;36(3):303.
11. Fouzia B, Damle AS. Prevalence and Characterization of Extended Spectrum Beta-Lactamase Production in Clinical Isolates of Klebsiella pneumonia. J Med Microbiology Diagn 2015; 4: 182.
12. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty Sixth Informational Supplements. CLSI Document M100-S28. Wayne, PA: Clinical and Laboratory Standards Institute. 2018.