Prevalence of Antimicrobial Resistant Pathogens in Severe Sepsis and Septic Shock Patients

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ABSTRACT

Emergence of antimicrobial resistance is turning to be a life threatening problem in the treatment of patients with severe sepsis and septic shock. Many organisms display a multidrug resistant (MDR) pattern, which limits the treatment options. **Aim:** The purpose of this study was to assess the prevalence of pathogens including the resistant types causing severe sepsis and septic shock as well as their antimicrobial resistance patterns. **Methods:** This was a prospective, observational study conducted at emergency department of a tertiary care hospital. A total of 293 bacterial isolates obtained during April 2015-16 inclusive, from severe sepsis and septic shock patients underwent susceptibility testing. **Results:** *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Candida albicans* and *Candida non-albicans* were the most common organisms.With respect to the specimen source, majority of the isolates (38.6%) were obtained from urine specimens followed by blood (27%), skin/soft tissue specimen (16.4%) and respiratory specimen (15%). *Escherichia coli* showed good susceptibility to amikacin, meropenem and piperacillin-tazobactam. *Klebsiella pneumoniae* showed high rates of resistance to all the tested antimicro-

bials except colistin. The MDR phenotype occurred in 24.6% *Escherichia coli*, 30.4% *Pseudomonas aeruginosa*, 56.4% *Klebsiella pneumoniae* and 87.5% *Acinetobacter baumannii*. No resistance was seen among fungal isolates. **Conclusion:** Our results highlight the high prevalence of gram negative organisms among severe sepsis and septic shock patients in south India. Strict implementation of sepsis guidelines, right antibiotic selection and dosage will help in preventing the development of resistance. **Key words:** Antimicrobials, Gram negative, Gram positive, Resistance, Septic shock, Severe sepsis.

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INTRODUCTION

The emergence of antimicrobial resistance is increasing at an alarming rate worldwide[1] and is becoming a serious threat to public health.[2-3] India can face dire consequences since it is the world's largest consumer of antibiotics.[4] Antibiotic overuse, inappropriate prescribing, extensive uses in poultry, poor infection control, have all contributed to the above phenomenon.[2,5] The emergence of multidrug resistance (MDR) especially in gram negative microbes like *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* has left clinicians with fewer treatment options, contributed to more healthcare resources and worst clinical outcomes.[2,6-7] Sepsis, resulting due to impairment of host defences to infection,[8-9] requires prompt implementation of antibiotic therapy to reduce morbidity and mortality.[10] Epidemiological studies have reported an increase in the incidence of severe sepsis and septic shock[11-12] with even gram positive and fungal organisms being the causative agents.[13] Thus, it is essential to have knowledge about the local epidemiological pat tern among the severe sepsis and septic shock patients since the organisms causing sepsis may not only vary based on regions but also change over time. Our study aims to assess the microbiological and susceptibility patterns of isolates obtained from severe sepsis and septic shock patients.

MATERIALS AND METHODS

This was a prospective, observational study conducted at our hospital from April 2015-16. Adut patients with a diagnosis of severe sepsis/septic shock were included in the study cohort, and only the first episode of severe sepsis or septic shock was counted. This study was approved by the institutional review board of the hospital (Thesis review committee/Pharma/2015/20). Being an observational study without any direct patient contact, the need for informed consent was waived. We used a case report form to collect data. In regard to microbiology results, cultures without any significant growth after the standard incubation period allotted for a particular specimen or cultures growing contaminants were classified as negative culture. A total of 293 isolates were recovered from the 250 enrolled patients. The clinical specimens included blood, urine, skin/soft tissue, and respiratory specimens. The isolates were identified by Vitek 2 Compact system (Biomerieux, France). All laboratory methods followed standard protocols. The susceptibility testing was done by both Kirby Bauer's disc diffusion and broth dilution methods as per Clinical and Laboratory Standards Institute (CLSI) guidelines.

RESULTS

Patient demographics and specimen types

In the present study, clinical specimens of 250 adult patients [203 severe sepsis and 47 septic shock] from our tertiary care hospital were evaluated. The patient age distribution was as follows: 18 to 40 years, 10.6% (31/293); 41 to 60 years, 40.6% (119/293); 61 to 80 years, 41.3% (121/293); >80 years, 7.5% (22/293). Out of 293 isolates, 204 were from males. One hundred and seventy eight (147 severe sepsis and 31 septic shock) patients had a positive culture. Majority of the patients [44.8% (112/250)] suffered from monomicrobial infection, while 66 patients (26.4%) suffered from polymicrobial infections. One hundred and thirty one

patients had a single site infection while 47 patients had multiple site infections. Overall, 293 isolates were obtained (240 isolates from severe sepsis patients and 53 isolates from septic shock patients). With regard to specimen source, 38.6% (113/293) of the organisms were obtained from urine specimens, 27% (79/293) from blood, 16.4% (48/293) from skin/soft tissue specimens, and 15% (44/293) from respiratory specimens. The remaining organisms were obtained from ascitic/peritoneal fluid, synovial fluid and bone.

Most common microorganisms isolated

In the present study, gram negative organisms were predominant (177/293, 60.4%) followed by gram positive (68/293, 23.2%) and fungi (48/293, 16.4%). Overall, the most common isolates were *Escherichia coli* (20.8%), *Klebsiella pneumoniae* (18.8%), *Pseudomonas aeruginosa* (7.8%), *Candida albicans* (6.1%) and *Candida non-albicans* (5.8%). The most common isolates among severe sepsis patients were *Escherichia coli* (52/240, 21.7%), *Klebsiella pneumoniae* (44/240, 18.3%), *Pseudomonas aeruginosa* (21/240, 8.8%), *Candida albicans* (15/240, 6.3%), *Candida non-albicans* (14/240, 5.8%). The most common isolates among septic shock patients were *Klebsiella pneumoniae* (11/53, 20.8%), *Escherichia coli* (9/53, 17%), *Candida albicans* (3/53, 5.7%), *Candida non-albicans* (3/53, 5.7%) and *Candida tropicalis* (3/53, 5.7%). The distribution of prevalent microbial species were found to be similar between patients with severe sepsis and septic shock.

Most common microorganisms isolated from specimen site

Overall, 113 isolates were obtained from urine specimens (91 isolates from severe sepsis patients and 22 isolates from septic shock patients). Among urinary tract specimens, *Escherichia coli* (20.4%), *Klebsiella pneumoniae* (18.6%) and *Candida non-albicans* (13.3%) were the predominant organisms. In patients with severe sepsis, *Klebsiella pneumoniae* (19/91, 20.9%) was the main isolate from urine specimen followed by *Escherichia coli* (18/91, 19.8%) and *Candida non-albicans* (12/91, 13.2%). The main isolates from the urine specimen of septic shock patients were *Escherichia coli* (5/22, 22.7%), *Candida albicans* (3/22, 13.6%) and *Candida non-albicans* (3/22, 13.6%).

Within the blood specimens, 79 isolates were obtained (65 isolates from severe sepsis patients and 14 isolates from septic shock patients). The predominant isolates were *Escherichia coli* (30.4%), *Klebsiella pneumoniae* (20.3%) and *Staphylococcus aureus* (8.9%). In patients with severe sepsis, the main isolate from blood specimen was *Escherichia coli* (21/65, 32.3%) followed by *Klebsiella pneumoniae* (11/65, 16.9%). The main isolates

from the blood specimen of septic shock patients were *Klebsiella pneumoniae* (5/14, 35.7%) and *Escherichia coli* (3/14, 21.4%).

With regard to skin/soft tissue specimens, 48 isolates were obtained (40 isolates from severe sepsis patients and 8 isolates from septic shock patients). *Escherichia coli* (16.7%), *Klebsiella pneumoniae* (10.4%) and *Pseudomonas aeruginosa* (8.3%) were the most commonly isolated organisms. In patients with severe sepsis, *Escherichia coli* (8/40, 20%) and *Pseudomonas aeruginosa* (4/40, 10%) were the common isolates whilst in septic shock patients, the predominant isolate was *Klebsiella pneumoniae* (2/8, 25%).

A total of 44 isolates were obtained from respiratory specimens (39 isolates from severe sepsis patients and five isolates from septic shock patients). In patients with severe sepsis, *Klebsiella pneumoniae* (10/39, 25.6%), *Candida albicans* (9/39, 23.1%) and *Pseudomonas aeruginosa* (9/39, 23.1%) were the most common organisms isolated. The organisms isolated from the respiratory specimen of septic shock patients were *alpha haemolytic streptococci*, *Candida tropicalis*, *Candida glabrata*, *Chromobacterium violaceum* and *Acinetobacter baumannii* (1/5, 20% each).

Antimicrobial susceptibility

Antimicrobial resistance rates for the most common gram negative organisms are listed in Table 1. *Escherichia coli* showed high resistance against amoxicillin-clavulanate, ampicillin-sulbactam, ceftriaxone and ciprofloxacin. However, it showed good susceptibility to amikacin, meropenam and piperacillin-tazobactam. More than 60% of the isolated *Klebsiella pneumoniae* strains were resistant to most of the antibiotics tested. *Pseudomonas aeruginosa* showed high resistance to ciprofloxacin (47.8%) and gentamicin (47.8%). *Acinetobacter baumannii* showed ≥75% resistance to amikacin, ampicillin-sulbactam, ciprofloxacin, cotrimoxazole, gentamicin, meropenam and piperacillin-tazobactam.

Resistance rates for the most common gram positive organisms are listed in Table 2. *Staphylococcus aureus* showed high resistance to penicillin G (83.3%) and ciprofloxacin (100%). *Enterococcus faecium* showed 100% resistance to ampicillin, amoxicillin, erythromycin, ciprofloxacin, ofloxacin and penicillin G.

Multi drug resistance (MDR)

Overall, 47 patients (41 severe sepsis and 6 septic shock) suffered from MDR infection. Out of the *Klebsiella pneumoniae* isolates tested, 56.4% (31/55) showed MDR. The MDR phenotype occurred in 24.6% (15/61) of *Escherichia coli* and 30.4% (7/23) of *Pseudomonas aeruginosa*. *Acinetobacter baumannii* showed high rates of MDR (87.5%). In the

Table 1: Resistance rates for the top five gram negative organisms.

Organism	AMI	AMOX-CLAV	AMP-SULB	CEFTR	CIPRO	COLI	COTRI	GENT	MER	PIPT	TIG
Escherichia coli	3/59 (5.1)	50/58 (86.2)	43/57 (75.4)	44/60 (73.3)	41/57 (71.9)	-	26/60 (43.3)	24/58 (41.4)	1/15 (6.7)	5/43 (11.6)	-
Klebsiella pneumoniae	28/51 (54.9)	40/52 (76.9)	34/54 (63)	34/54 (63)	33/51 (64.7)	1/30 (3.3)	33/54 (61.1)	33/53 (62.3)	21/34 (61.8)	28/36 (77.8)	6/11 (54.5)
Pseudomonas aeruginosa	9/22 (40.9)	-	-	-	11/23 (47.8)	1/9 (11.1)	-	11/23 (47.8)	5/13 (38.5)	3/15 (20)	-
Acinetobacter baumannii	6/8 (75)	-	6/8 (75)	-	7/8 (87.5)	-	6/8 (75)	6/7 (85.7)	6/7 (85.7)	7/8 (87.5)	1/4 (25)
Proteus mirabilis	-	-	1/1 (100)	1/1 (100)	-	-	-	-	-	-	-

AMI = amikacin, AMOX-CLAV = amoxicillin-clavulanate, AMP-SULB = ampicillin-sulbactam, CEFTR = ceftriaxone, CIPRO = ciprofloxacin, COLI = colistin, COTRI = cotrimoxazole, GENT = gentamicin, MER = meropenam, PIPT = piperacillin-tazobactam, TIG = tigecycline.

Table 2: Resistance rates for the top five gram positive organisms.

Organism	AMP/ AMOX	CIPRO	CLIND	CLOX/ OXA	COTRI	ERY	NITRO	OFLO	PEN G	TET
Staphylococcus aureus	-	1/1 (100)	2/6 (33.3)	5/12 (41.7)	4/12 (33.3)	1/2 (50)	-	-	10/12 (83.3)	-
Enterococcus species	7/9 (77.8)	3/3 (100)	-	-	-	3/3 (100)	6/8 (75)	4/4 (100)	6/8 (75)	1/7 (14.3)
Enterococcus faecalis	2/9 (22.2)	2/2 (100)	1/1 (100)	-	-	3/4 (75)	1/5 (20)	3/4 (75)	2/10 (20)	-
Enterococcus faecium	7/7 (100)	2/2 (100)	-	-	-	1/1 (100)	3/5 (60)	2/2 (100)	7/7 (100)	2/3 (66.7)
Streptococcus species	-	-	-	-	-	2/5 (40)	-	1/6 (16.7)	1/5 (20)	-

AMP/AMOX = ampicillin/amoxicillin, CIPRO = ciprofloxacin, CLIND = clindamycin, CLOX/OXA = cloxacillin/oxacillin, COTRI = cotrimoxazole, ERY = erythromycin, NITRO = nitrofurantoin, OFLO = ofloxacin, PEN G = penicillin G, TET = tetracycline.

present study, 79 patients (39 severe sepsis and 40 septic shock) expired. Of these 79 expired patients; 20 patients had negative culture, 29 patients had monomicrobial infection and 30 patients had polymicrobial infection. Of the 47 patients with MDR infection, 18 patients (12 severe sepsis and 6 septic shock) expired. Among the fungal isolates, no resistance was observed. Among the *Staphylococcus aureus* isolates, 38.5% (5/13) showed methicillin resistance.

DISCUSSION

During the period of 2015-16, the clinical samples of 250 severe sepsis and septic shock patients was analyzed for the presence of microorganisms. Traditionally, respiratory infections were considered to be the main cause of severe sepsis and septic shock.[12,14] However, in the present study, urinary tract was the major infectious site. Ours being a tertiary care hospital, majority of our patients were referred cases and had Foley's catheter on admission in our hospital. This could be one of the reason for the high proportion of urinary isolates observed in our study.

Previously, sepsis was considered to be related with gram negative bacteria only. However, some epidemiological studies have shown that even gram positive organisms and fungi could be common causes of sepsis.[13] In patients with severe sepsis and septic shock, prompt treatment of infection is the cornerstone of therapy and predictive of survival.[10] To select the right antimicrobial for the offending pathogen, knowledge of the causative organisms and their local antimicrobial susceptibility patterns is pivotal. In the present study, the vast majority of isolates were *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. High rates of MDR among the gram negative isolates were observed in the present study, which represents an increasing threat to patients. High resistance was seen among the *Escherichia coli* species against amoxicillin-clavulanate, ampicillin-sulbactam, ceftriaxone and ciprofloxacin. *Escherichia coli* is posing a threat as these resistant strains have the ability to transfer resistant determinants not only to their own strains but also to other bacteria present in the gastrointestinal tract. In our hospital setting, *Klebsiella pneumoniae* and *Acinetobacter baumannii* showed high rates of resistance to majority of the agents. Thus, the treatment options for MDR *Klebsiella pneumoniae* appears to be limited to combination therapy with some aminoglycosides, tigecycline and to older antimicrobial agents like colistin. *Acinetobacter baumannii* too appeared to have a high capacity to acquire antibiotic resistance. This may be because it has different mechanisms of resistance such as antimicrobial inactivating enzymes (eg., beta-lactamases), reduced access to bacterial targets (eg., altered porin channels), efflux pumps, aminoglycoside modifying enzymes etc.[15] Piperacillin-tazobactam and meropenem showed effectiveness against *Pseudomonas aeruginosa*.

CONCLUSION

Our results highlight the high prevalence of gram negative organisms among severe sepsis and septic shock patients in south India. Strict implementation of sepsis guidelines, right antibiotic selection and dosage will help in preventing the development of resistance.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

ABBREVIATIONS

CLSI: Clinical and Laboratory Standards Institute; **MDR:** Multidrug resistance.

SUMMARY

A high prevalence of gram negative organisms were seen, with *Escherichia coli* being the most common isolate. A high rate of multidrug resistance was observed especially in *Acinetobacter baumannii* and *Klebsiella pneumoniae*.

REFERENCES

1. Ferri M, Ranucci E, Romagnoli P, Giaccone V. Antimicrobial resistance: A global emerging threat to public health systems. Crit Rev Food Sci Nutr. 2017;57(13):2857-76.
2. Tanwar J, Das S, Fatima Z, Hameed S. Multidrug resistance: an emerging crisis. Interdiscip Perspect Infect Dis. 2014;2014:541340.
3. Michael CA, Dominey-Howes D, Labbate M. The antimicrobial resistance crisis: causes, consequences and management. Front Public Health. 2014;2:145.
4. Laxminarayan R, Chaudhury RR. Antibiotic resistance in India: Drivers and opportunities for action. PLoS Med. 2016;13(3):e1001974.
5. Fair RJ, Tor Y. Antibiotics and bacterial resistance in the 21st century. Perspect Medicin Chem. 2014;6:25-64.
6. Khety Z, Mohanta G, Jain S, Dawoodi S. Changing antimicrobial resistance pattern of isolates from an ICU over a 3 year period. J Assoc Physicians India.

2017;65(2):13-6.

7. Cai B, Echols R, Magee G, Arjona Ferreira JC, Morgan G, Ariyasu M, *et al.* Prevalence of carbapenem-resistant gram-negative infections in the United States predominated by *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. Open Forum Infect Dis. 2017;4(3):176.

8. Ozdemir V, Hekim N. Innovation management? Orienting sepsis R and D and technology transfer towards stratified medicine. E Bio Med. 2016;6:8-9.

9. Mayr FB, Yende S, Angus DC. Epidemiology of severe sepsis. Virulence. 2014;5(1):4-11.

10. Mok K, Christian MD, Nelson S, Burry L. Time to administration of antibiotics among inpatients with severe sepsis or septic shock. Can J Hosp Pharm. 2014;67(3):213-9.

11. Babu M, Menon VP, Devi UP. Epidemiology and outcome among severe sepsis and septic shock patients in a South Indian tertiary care hospital. Int J Pharm Pharm Sci. 2017;9(5):256-9.

12. Blanco J, Bombin AM, Sagredo V, Taboada F, Gandia F, Tamayo L, *et al.* Incidence, organ dysfunction and mortality in severe sepsis: a Spanish multicenter study. Crit Care. 2008;12(6):R158.

13. Martin GS. Sepsis, severe sepsis and septic shock: Changes in incidence, pathogens and outcomes. Expert Rev Anti Infect Ther. 2012;10(6):701-6.

14. Huang CT, Tsai YJ, Tsai PR, Yu CJ, Ko WJ. Epidemiology and outcome of severe sepsis and septic shock in surgical intensive care units in Northern Taiwan. Medicine (Baltimore). 2015;94(47):e2136.

15. Manchanda V, Sanchaita S, Singh N. Multidrug resistant acinetobacter. J Glob Infect Dis. 2010;2(3):291-304.