ORIGINAL ARTICLE

Bacteriological Profile and Antimicrobial Susceptibility Pattern of Intra-abdominal Infections: A Study from a Tertiary Care Hospital of North India

[1]Ritu Garg, [2]Varsha A Singh

ABSTRACT

Background and objectives: Intra-abdominal infections (IAIs) are associated with significant morbidity and mortality. Pathogenic isolates and emerging resistance to commonly used antimicrobials have been a matter of concern in IAIs. In the present study, bacteriological profile and antimicrobial susceptibility of isolates from IAIs were investigated.

Materials and methods: A total of 145 samples (ascitic fluids, n = 56; bile, n = 20; and pus, n = 36) were collected from suspected IAI of patients reporting to the hospital and cultured. Identification of the isolates was done using standard identification protocol. Antimicrobial susceptibility was performed by Kirby-Bauer disk diffusion method and interpretation was done according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Results: Of 145 samples, 112 were culture positive and 33 were sterile. Gram-negative organisms (n = 85) outnumbered the Gram-positive organisms (n = 27). Among the Gram-negative organisms, *Escherichia coli* (n = 31) was the most commonly isolated organism followed by *Klebsiella* sp. (n = 19), *Acinetobacter* sp. (n = 14), *Pseudomonas* sp. (n = 10), *Proteus* sp. (n = 5), *Citrobacter* sp. (n = 3), and *Enterobacter* sp. (n = 3). Among the Gram-positive bacteria, the most common organism was *Staphylococcus aureus* (n = 19) followed by *Enterococcus faecalis* (n = 8). Gram-negative bacilli showed significant resistance to almost all of the commonly used antibiotics. The rate of methicillin-resistant *S. aureus* (MRSA) was 36.84%.

Conclusion: Prompt starting of empirical antimicrobials based on the local susceptibility pattern, followed by modification of treatment in accordance with the antimicrobial susceptibility report can significantly reduce the morbidity and the mortality associated with IAIs.

Keywords: Emerging resistance, Empirical antimicrobials, Intra-abdominal infections.

[1]Associate Professor, [2]Professor and Head

[1,2]Department of Microbiology, Maharishi Markandeshwar Institute of Medical Sciences and Research, Ambala, Haryana India

Corresponding Author: Ritu Garg, Associate Professor Department of Microbiology, Maharishi Markandeshwar Institute of Medical Sciences and Research, Ambala, Haryana, India, e-mail: dr_rittu07@yahoo.co.in

INTRODUCTION

Intra-abdominal infections involve extensive variety of pathological conditions ranging from uncomplicated appendicitis to fecal peritonitis.[1] Intra-abdominal infections are classified into uncomplicated IAIs and complicated IAIs.[2,3] They are further classified into community-acquired IAIs and hospital-acquired IAIs. Uncomplicated IAIs involve a single organ and can be easily managed by surgical resection and antibiotics, while complicated IAIs involve more than one organ, including peritoneum, and leads to either local or diffuse peritonitis. Complicated IAIs remain an important cause of patient morbidity and are frequently associated with poor clinical prognosis. Hospital-acquired IAIs are associated with higher mortality rates because of compromised patient's immunity due to underlying illness and infections with multidrug-resistant organisms.[4] Antimicrobial treatment plays a vital role for management of critically ill patients with IAIs.[2] Initially, empirical therapy should be started based on the most frequently isolated organisms and according to the local pattern of antibiotic resistance, which should be modified to specific antimicrobials after receiving the microbiology report of antimicrobial susceptibility of the isolated organism.[4] Hence, accurate and timely identification of pathogenic organisms along with their susceptibility testing requires the attention of microbiologists along with the clinicians' diagnosis.

The threat of antimicrobial resistance to commonly used antibiotics is the major challenge faced by the clinicians nowadays in treating IAIs. As a result, growing emergence of multidrug resistance, limited availability of newer antibiotics, and scarcity of local data on antimicrobial resistance pattern of IAIs create a void for the management of IAIs. By keeping in mind the above lacunae, the present study was planned to determine the bacteriological profile and antimicrobial susceptibility pattern of isolates from IAIs.

MATERIALS AND METHODS

Patients and Samples

The present cross-sectional study was conducted to determine the bacteriological profile and antimicrobial

susceptibility pattern of isolates from samples obtained from IAIs in the Department of Microbiology, Maharishi Markandeshwar Institute of Medical Sciences & Research, Ambala, Haryana, India. Ethical clearance for the study was taken from the Institutional Ethical Committee. The inclusion criterion was samples from clinically suspected cases of IAIs of all ages and the exclusion criterion was noninclusion of samples from patients having infections other than IAIs.

Processing of Samples

A total of 145 samples, viz., ascitic fluid, bile, and pus, were obtained from patients suffering from suspected IAIs like peritonitis, cholelithiasis, appendicitis, pancreatitis, and liver abscess. All the samples were cultured on blood agar and MacConkey agar and incubated at 37°C for 18 to 24 hours. Gram staining was performed on each sample. Identification of the isolates was done using standard identification protocol and other relevant biochemical tests as appropriate for the isolates.[5-7]

Antibiotic Susceptibility Tests

Antibiotic susceptibility of the isolates was performed by Kirby-Bauer disk diffusion method and interpreted according to the CLSI guidelines, except for tigecycline for which European Committee on Antimicrobial Susceptibility Testing guidance document was followed.[8-10] Antibiotic susceptibility testing for Gram-negative organisms was put up using gentamicin, amikacin, ciprofloxacin, levofloxacin, cotrimoxazole, ceftriaxone, cefotaxime, imipenem, amoxicillin-clavulanic acid, piperacillin-tazobactam, and colistin.

Antimicrobial susceptibility testing for Gram-positive cocci was put up as follows:

- *Staphylococcus aureus*: Methicillin resistance for *S. aureus* was detected by cefoxitin disk diffusion method as recommended by CLSI guidelines,[9] and *S. aureus* showing resistance to cefoxitin was considered as MRSA. This MRSA was also tested for antimicrobial susceptibility toward cotrimoxazole, erythromycin, clindamycin, ciprofloxacin, amikacin, vancomycin, linezolid, dalfopristin/quinupristin, and tigecycline. Methicillin-sensitive *S. aureus* (MSSA) was tested for antibiotic susceptibility toward ampicillin, amoxicillin-clavulanic acid, cotrimoxazole, erythromycin, clindamycin, ciprofloxacin, amikacin, vancomycin, and linezolid.
- *Enterococcus faecalis*: Antibiotic susceptibility of *E. faecalis* was done using penicillin, ampicillin, erythromycin, tetracycline, chloramphenicol, vancomycin, linezolid as well as high-level gentamicin (120 μg) and high-level

streptomycin (300 μg). Vancomycin-resistant *Enterococci* were detected based on disk diffusion results using vancomycin disk (30 μg) as per CLSI guidelines.[9] Vancomycin-resistant *E. faecalis* was subjected to linezolid and tigecycline.

RESULTS

Of 145 samples collected from suspected IAIs, 112 showed positive culture, whereas the remaining 33 were sterile, showing a positivity rate of 77.24%. Of 112 culture-positive samples, 56 were ascitic fluids, 20 bile samples, and 36 pus samples (Table 1). Both Gram-positive and Gram-negative organisms were isolated from the processed samples. Gram-negative organisms (n = 85) outnumbered the Gram-positive organisms (n = 27). Among the Gram-negative organisms, *E. coli* (n = 31) was the most commonly isolated organism followed by *Klebsiella* sp. (n = 19), *Acinetobacter* sp. (n = 14), *Pseudomonas* sp. (n = 10), *Proteus* sp. (n = 5), *Citrobacter* sp. (n = 3), and *Enterobacter* sp. (n = 3). Among the Gram-positive bacteria, *S. aureus* (n = 19) was the most common isolate, followed by *E. faecalis* (n = 8; Table 2).
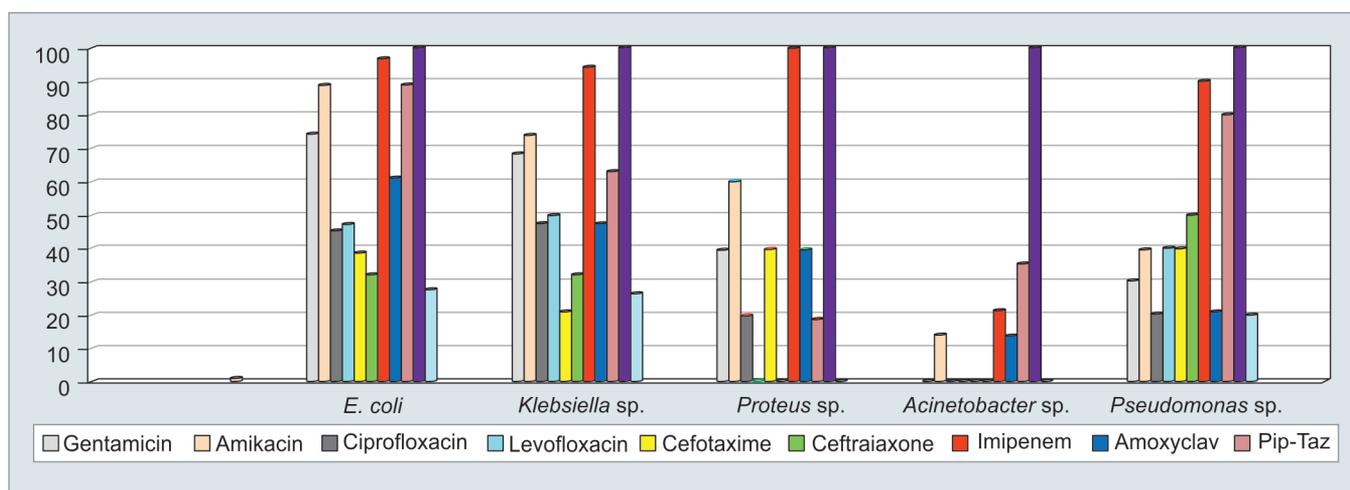
All the Gram-negative bacilli showed substantial resistance to almost all of the commonly used antibiotics. The most commonly isolated bacilli, viz., *E. coli*, showed the least sensitivity to cotrimoxazole (29.03%) followed by ceftriaxone (32.25%), cefotaxime (38.70%), ciprofloxacin (45.16%), amoxicillin-clavulanic acid (61.29%), gentamicin (74.19%), amikacin (89.09%), piperacillin-tazobactam (89.09%), and imipenem (96.77%). *Klebsiella* sp. also showed the same pattern of sensitivity. *Citrobacter* sp. and *Enterobacter* sp. showed 100% sensitivity to all the antimicrobials tested. Among nonlactose fermenters, *Acinetobacter* sp. was found to be the most resistant organism. All the 14 isolates of *Acinetobacter* sp. showed multidrug resistance and only 21.42% isolates showed sensitivity to imipenem. On the contrary, *Pseudomonas* sp. showed better sensitivity as compared with *Acinetobacter* sp. Graph 1 shows antimicrobial sensitivity pattern of Gram-negative bacilli.

Table 1: Samples collected from various GI infections

Gastrointestinal infections	No. of cases	Samples received	No. of samples
Peritonitis	60	Ascitic fluid	53
		Pus	7
Cholelithiasis	19	Bile	19
Appendicitis	13	Pus	13
Pancreatitis	9	Ascitic fluid	3
		Pus	5
		Bile	1
Liver abscess	11	Pus	11
Total	*112*		*112*

Table 2: Organisms isolated from various GI infections

| Gastrointestinal infection					Organisms isolated				
	E. coli	Klebsiella sp.	Proteus sp.	Citrobacter sp.	Enterobacter sp.	Pseudomonas sp.	Acinetobacter sp.	S. aureus	Enterococcus sp.
Peritonitis	15	11	2	3	3	4	7	9	6
Cholelithiasis	6	2	1	0	0	3	3	4	0
Appendicitis	3	3	0	0	0	1	2	3	1
Pancreatitis	4	1	1	0	0	1	0	2	0
Liver abscess	3	2	1	0	0	1	2	1	1
Total	*31*	*19*	*5*	*3*	*3*	*10*	*14*	*19*	*8*

Graph 1: Percentage of antimicrobial susceptibility of Gram-negative bacilli isolated from IAIs

Among the Gram-positive bacteria, *S. aureus* was the most common organism isolated. The rate of methicillin resistance was 36.84% (7/19) in *S. aureus.* All the MRSA showed 100% sensitivity to vancomycin, linezolid, dalfopristin/quinupristin, and tigecycline and 71.4% to cotrimoxazole, 57.14% each to amikacin and clindamycin, 28.57% to ciprofloxacin, and 42.8% to erythromycin.

All (100%) MSSA were sensitive to vancomycin and linezolid and 91.66% each to amikacin and clindamycin. Susceptibility was 75% toward cotrimoxazole, 58.33% for erythromycin, and 50% for ciprofloxacin. The susceptibility pattern of both MSSA and MRSA is shown in Graph 2.

All the eight isolates of *E. faecalis* showed susceptibility to linezolid, while three of eight isolates showed resistance to vancomycin. Sensitivity to penicillin, ampicillin, erythromycin and tetracycline was very low, whereas 50% *E. faecalis* isolates showed susceptibility to chloramphenicol.

A high-level resistance to aminoglycosides, i.e., 25% to gentamicin (120 μg) and 37.5% to streptomycin (300 μg), was seen in three of the eight isolates. Two of the three showed combined resistance to both the high-level aminoglycosides. Three isolates showed resistance to vancomycin, but none of the isolates showed resistance to linezolid.
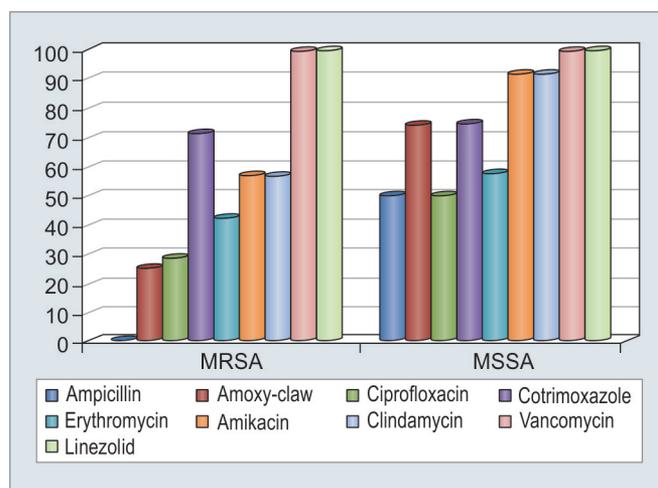
Graph 2: Antibiotic susceptibility patterns of *Staphylococcus aureus* isolated from IAIs

Vancomycin-resistant *Enterococci* were tested against linezolid and tigecycline and showed 100% susceptibility to both linezolid and tigecycline.

DISCUSSION

The emergence of resistance to routinely used antibiotics and even to newer antibiotics has made the treatment of IAIs a real challenge for clinicians. Accurate laboratory identification of the isolates along with routine sensitivity

testing is needed. Proper collection and transport of specimens is a great challenge. Furthermore, data on local pattern of susceptibility of pathogens can help in guiding the treatment of these pathogens. Hence, clinicians and microbiologists working in tandem can go a long way in decreasing the mortality due to IAIs.

The rate of isolation of pathogenic organisms from our study was 112/145 (77.24%). Both Gram-negative bacilli and Gram-positive cocci were isolated. Among the Gram-negative bacilli, the major pathogens involved belonged to the family Enterobacteriaceae. These observations were also made by Sartelli et al.[1] Among the family Enterobacteriaceae, *E. coli* was the most common organism isolated. Studies done by Kurup et al[11] and Hawser et al[12] reported similar results. Apart from *E. coli,* organisms isolated were *Klebsiella* sp., *Proteus* sp., *Citrobacter* sp., and *Enterobacter* sp., while among the nonlactose fermenters *Acinetobacter* sp. was comparatively more common than *Pseudomonas* sp. These results are in concordance with the study done by Saad et al.[2] Among the Gram-positive bacteria, *S. aureus* was more commonly isolated than *E. faecalis*, compared with the study by Shree et al.[13]

In the present study, antimicrobial susceptibility data obtained showed that most of the Gram-negative bacilli were multidrug resistant to the routinely used antibiotics, which is an alarming situation. A high rate of antimicrobial resistance to third-generation cephalosporins was also observed. Combination antibiotics like amoxicillin/clavulanic acid also showed 61.29% sensitivity to *E. coli*, 47.36% to *Klebsiella* sp., and 20% to *Proteus* sp., while piperacillin/tazobactam showed 89.09% to *E. coli*, and 63.15% to *Klebsiella* sp. These findings are similar to the study done by Saad et al.[2] Better than best susceptibility was shown by imipenem except with *Acinetobacter* sp. Studies by Hawser et al[12] and Oteo et al[14] also showed similar findings. However, in our study, no resistance toward colistin was observed.

The rate of MRSA was 36.84%, which is in comparison with the study by Shree et al[13] and Datta et al.[15] The most active agents against MRSA were vancomycin, linezolid, quinupristin/dalfopristin, and tigecycline that showed 100% susceptibility. Also, MSSA showed 100% sensitivity to vancomycin and linezolid. Our observations are in concordance with the studies done by Datta et al[15] and Montravers et al.[16]

In *E. faecalis*, increased resistance to various antimicrobials was observed as reported by Akhter et al[17] and Chaudhary et al.[18] In our study, 37.5% showed high-level resistance to gentamicin and/or streptomycin. Combined resistance to both the high-level aminoglycosides, viz., gentamicin and streptomycin, was seen in 25% of cases. Three isolates showing high-level resistance to aminoglycosides

also showed resistance to vancomycin. Our study results are in comparison to the study done by Huidrom et al.[19] But all the vancomycin-resistant *E. faecalis* showed 100% susceptibility to linezolid and tigecycline. These findings are in agreement with previous studies.[20-22]

CONCLUSION

Cautious use of antibiotics for empirical therapy followed by modification of treatment after receiving antimicrobial susceptibility report can significantly reduce the morbidity and the mortality related to IAIs.

REFERENCES

1. Sartelli M, Catena F, Ansaloni L, Leppaniemi A, Taviloglu K, van Goor H, Viale P, Lazzareschi DV, Coccolini F, Corbella D, et al. Complicated intra-abdominal infections in Europe: a comprehensive review of the CIAO study. World J Emerg Surg 2012 Nov;7(1):36.
2. Saad U, Anwar S, Kahara UZ, Siddiqui M, Saeed H. Antimicrobial susceptibility of intra-abdominal infection isolates from a tertiary care hospital in Karachi. J Ayub Med Coll Abbottabad 2016 Jul-Sep;28(3):568-571.
3. Mazukim JE, Solomon JS. Intra-abdominal infections. Surg Clin North Am 2009 Apr;89(2):421-437.
4. Sartelli M, Catena F, Ansaloni L, Moore E, Malangoni M, Velmahos G, Coimbra R, Koike K, Leppaniemi A, Biffl W, et al. Complicated intra-abdominal infections in a worldwide context: an observational prospective study (CIAOW Study). World J Emerg Surg 2013 Jan;8(1):3-7.
5. Colle JG, Miles RS, Watt B. Tests for identification of bacteria. In: Collee JG, editors. Mackie and McCartney Practical medical microbiology. 14th ed. Edinburgh: Churchill Livingstone; 1996. pp. 151-179.
6. Ananthanarayan R, Jayaram Paniker CK. Urinary tract infections. In: Kapil A, editor. Text book of microbiology. 9th ed. Hyderabad, India: University Press; 2013. p. 671.
7. Sastry AS, Bhat SK. Clinical microbiology (infective syndrome). In: Essentials of medical microbiology. 1st ed. Delhi: JAYPEE Publications; 2016. pp. 585-588.
8. Bauer AW, Kirby WMM, Sherris JC, Jurck M. Antibiotic susceptibility testing by a standardized single disc method. Am J Clin Pathol 1966 Apr;45(4):493-496.
9. CLSI. Performance standards for antimicrobial susceptibility testing; 23rd Informational Supplement. CLSI Document M100-S23, Wayne PA: Clinical and Laboratory Standards Institute; 2013.
10. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint tables for interpretation of MICs and zone diameters, version 1.1. Stockholm, Sweden: EUCAST; 2010.
11. Kurup A, Liau KH, Ren J, Lu MC, Navarro NS, Farooka MW, Usman N, Destura RV, Sirichindakul B, Tantawichien T, et al. Antibiotic management of complicated intra-abdominal infections in adults: The Asian perspective. Ann Med Surg 2014 Aut;3(3):85-91.
12. Hawser SP, Badal RE, Bouchillon SK, Hoban DJ. Antibiotic susceptibility of intra-abdominal infection isolates from Indian hospitals during 2008. J Med Microbiol 2010 Sep; 59(9):1050-1054.

13. Shree N, Arora BS, Mohil RS, Kasana D, Biswal I. Bacterial profile and patterns of antimicrobial drug resistance in intra-abdominal infections: Current experience in a teaching hospital. Indian J Pathol Microbiol. 2013 Jan;56(4):388-392.

14. Oteo J, Lazaro E, de Abajo FJ, Baquero F, Campos J. Antimicrobial resistant invasive *Escherichia coli*, Spain. Emerg Infect Dis 2005 Apr;11(4):546-553.

15. Datta P, Gulati N, Singla N. Vasudeva HR, Bala K, Chander J, Gupta V. Evaluation of various methods for the detection of methicillin-resistant *Staphylococcus aureus* strains and susceptibility patterns. J Med Microbiol 2011 Nov;60(Pt 11):1613-1616.

16. Montravers P, Lepape A, Dubreuil L, Gauzit R, Pean Y, Benchimol D, Dupont H. Clinical and microbiological profiles of community acquired and nosocomial intra-abdominal infections: Result of the French prospective, observational EBIIA study. J Antimicrob Chemother 2009 Apr;63(4): 785-794.

17. Akhter J, Ahmed S, Anwar S. Antimicrobial Susceptibility Patterns of *Enterococcus* species Isolated from urinary tract infections. Bangladesh J Med Microbiol 2014;8(1):16-20.

18. Chaudhary U, Shamma M, Yadav A. Antimicrobial susceptibility patterns of common and unusual Enterococcus species isolated from clinical specimens. J Infect Dis Antimicrob Agents 2007 Jan;24(2):55-62.

19. Huidrom S, Narayanaswamy G, Dadlani R. Detection of high-level aminoglycoside resistant pattern of Enterococci isolated from urine samples at a tertiary care hospital in Bengaluru. Ann Trop Med Public Health 2016 May;9(3):165-169.

20. Gupta V, Singla N, Behl P, Sahoo T, Chander J. Antimicrobial susceptibility pattern of vancomycin resistant enterococci to newer antimicrobial agents. Indian J Med Res 2015 Apr;141(4):483-486.

21. Chitnis S, Katara G, Hemvani N, Pareek S, Chitnis DS. In vitro activity of daptomycin & linezolid against methicillin resistant *Staphylococcus aureus* & vancomycin resistant enterococci isolated from hospitalized cases in Central India. Indian J Med Res 2013 Jan;137(1):191-196.

22. Manoharan A, Chatterjee S, Madhan S, Mathai D. Evaluation of tigecycline activity in clinical isolates among Indian medical centers. Indian J Pathol Microbiol 2010 Oct-Dec;53(4):734-737.