Indian Heart Journal 73 (2021) 704–710

Contents lists available at ScienceDirect

Indian Heart Journal

journal homepage: www.elsevier.com/locate/ihj

Original Article

Changing microbiological profile and antimicrobial susceptibility of the isolates obtained from patients with infective endocarditis – The time to relook into the therapeutic guidelines

Rama Gupta [c], Vandana Kaushal [c], Abhishek Goyal [a], Pawan Kumar [b], Dinesh Gupta [b], Rohit Tandon [a], Aashita Mahajan [d], Sonaal Singla [a], Gurbhej Singh [a], Bhupinder Singh [a], Shibba Takkar Chhabra [a], Naved Aslam [a], Gurpreet S. Wander [a], Veenu Gupta [c], Bishav Mohan [a, *]

[a] Department of Cardiology, Dayanand Medical College and Hospital, Unit Hero DMC Heart Institute, Ludhiana, India
[b] Department of Medicine, Dayanand Medical College and Hospital, Ludhiana, India
[c] Department of Microbiology, Dayanand Medical College and Hospital, Ludhiana, India
[d] Dayanand Medical College and Hospital, Ludhiana, India

ARTICLE INFO

Article history:
Received 22 May 2021
Accepted 29 October 2021
Available online 1 November 2021

Keywords:
Infective endocarditis
Microbiological profile
Minimum inhibitory concentrations
Guidelines
Intravenous drug users

ABSTRACT

The microbiological profile, associated risk factors and demographic characteristics of patients with IE has changed in the recent times. In the present study, the antibiotic susceptibility profile of 66 isolates (40 from IDU and 26 from non IDU) recovered over a period of three years from the patients with definitive diagnosis of IE along with their absolute minimum inhibitory concentrations (MIC-μg/ml) was determined as per CLSI, 2017 guidelines. *Staphylococcus aureus* was found to be the predominant pathogen associated with IE out of which 90.2% isolates were MRSA, although none of the isolates were found resistant to vancomycin, teicoplanin, daptomycin and linezolid. *Pseudomonas aeruginosa* isolates were 100% susceptible to carbapenams, however variable resistance was observed against other antimicrobials. All Enterococci were found to be 100% susceptible to linezolid and daptomycin, whereas vancomycin resistant enterococci phenotype was observed in 25% of the Enterococcal isolates. A noticeable difference in the antimicrobial susceptibility profile and their MICs were observed in the present study, as compared to published literature across the globe and within the country. However, no statistically significant difference (λ^2 test, $p > 0.01$)in the AST pattern of isolates from IDU vs. Non IDU was observed. After reviewing the local antibiogram it seems that we need to have our own regional guidelines, which may partially replace the currently prevailing AHA/ESC guidelines.

1. Introduction

Infective endocarditis (IE) is defined as a microbial infection in the heart valves or endocardium or intracardiac devices. This condition is by and large caused by bacteria and rarely by fungi entering the bloodstream and infecting the heart. In developed countries, the incidence of endocarditis ranges from 2.6 to 7 cases per 100,000 populations per year, with a mortality of up to 10–30% at 30 days.[1,2]

The disease profile of IE has been continuously evolving ever since Osler's narrativeof infective endocarditis in the year 1985[3] and has been discussed extensively in many studies from industrialized world. The microbiological profile, associated risk factors and demographic characteristics of the patients withinfective endocarditis has substantially changed over the years due to increase in the use of intracardiac devices & intravenous catheters, increase in number of patients undergoing hemodialysis, increase in the number of intravenous drug abusers (IDU), increased number of patients undergoing immunosuppressive therapy. Another factor that has contributed to this change is increased survival of patients with congenital heart disease till their adulthood.[4,5] *Staphylococcus aureus* and group D Streptococci (enterococci) has substantially

* Corresponding author.
E-mail address: bishav_68@yahoo.co.in (B. Mohan).

https://doi.org/10.1016/j.ihj.2021.10.013

replaced the oral Streptococci as the most prevalent cause of infective endocarditis.[6,14] Although the microbiology of IE depends on whether it involves an native valve or prosthetic valve, whether the disease is hospital or community acquired, and whether the patient is intravenous drug abuser or Non IDU.[6,7] In intravenous drug abusers the predominant pathogens include *Staphylococcus aureus*, *Pseudomonas aeruginosa* and fungi.[5,8] Not much epidemiological data is available in this context from the developing countries including the Indian subcontinent.

In addition to these sequential epidemiological changes, major new findings from multiple diagnostic, prognostic, and therapeutic studies have been published since the last iteration of the American Heart Association (AHA) statement on diagnosis and management of IE complications in 2015.[2] These iterations are evidence based for diagnostic and treatment recommendations and emphasized the importance of unique characteristics of the infected vegetations, host immunity, unique pharmacokinetics/pharmacodynamics (PK/PD) of the antimicrobial agents. Nonetheless the local antimicrobial susceptibility pattern of the incriminated organisms needs to be considered for the treatment of any infectious disease. Therefore present study was under taken to study the changing microbiological profile and antibiotic susceptibility pattern of the isolates obtained from patients with definite diagnosis of infective endocarditis, in reference to the current guidelines for the management of infective endocarditis.

2. Methodology

This study was carried out in a super specialty tertiary care referral hospital. This was a retrospective study on the bacterial and fungal isolates obtained from all the patients admitted with definite diagnosis of infective endocarditis (IE) as per Modified Duke "definitive" criteria[5,9,10] during the period from January 2017–December 2019 (3 years). Patients with clinical suspicion of infective endocarditis who could not be categorized as definite infective endocarditis as per Duke's criteria were excluded from the study. Detailed Information was collected for each patient on his/her demographic profile, predisposing heart condition/disease, immunocompromised condition if any, any surgical intervention including dental/gynecological procedure in the recent past, presenting symptoms, complications and other relevant laboratory investigations. Transthoracic echocardiography (TTE) was performed on all the patients with suspicion of IE; however transesophageal echocardiogram (TEE) was performed only on patients with non-diagnostic TTE, suspected prosthetic valve endocarditis (PVE) or suspected cardiac mechanical complications. CT scan brain and abdominal sonography were carried out wherever necessary.[5]

Blood culture: Three sets of blood culture were taken from three different sites of the body (right cubital fossa, left cubital fossa and left wrist) at intervals over 24 h. All the blood specimens were collected taking all aseptic precautions and were processed as per standard procedure, using automated blood culture system BACTEC 9240, Biomerieux, USA.[5] The Clinical and Laboratory Standards Institute (CLSI) 2017 guidelines were used to interpret the susceptibility profile of the isolates based upon the minimum inhibitory concentration (MIC μg/ml) of the drugs obtained.[11] Quality control (QC) was performed using Escherichia coli ATCC 25922 Staphylococcus aureus ATCC25923 and P. aeruginosa ATCC 27853.

Statstical analysis: For comparing antimicrobial susceptibility profile of the isolates from IDU and Non IDU IE patients, Chi square ($\chi 2$) test was performed. A probability value (p value) less than 0.01 was considered statistically significant. All statistical calculations were done using SPSS (Statistical Package for the Social Science) SPSS 21 version statistical program for Microsoft Windows.

3. Results

A total of 133 patients fulfilling the modified Duke's criteria, based upon the demographic, clinical and laboratory investigations were included in the study. The present study is an continuation of the previous work, whereby demographic and clinical profile of these patients was discussed with special reference to the intravenous drug abusers.[5] A total of 66 isolates were obtained over a period of three years. These isolates included 41 isolates of *Staphylococcus aureus,* twelve isolates of *Pseudomonas aeruginosa*, four isolates of members of family enterobacteriacae (*E.coli* and *Klebsiella pneumoniae*-2 each), four isolates of *Enterococcus fecalis*, three isolates of Candia species (2 isolates of *Candida famata* and one isolate of *Candida tropicalis*) and one isolate each of *Achromobacter xylosoxidans* and *Streptococcus aglactiae* were obtained as has been listed in the previous study.

Antibiotic susceptibility pattern of these isolates was analyzed. The isolates were labeled as sensitive, intermediately sensitive and resistant to the various antibiotic tested, based upon their MIC breakpoints (μg/ml) defined in the CLSI, 2017 guidelines. The antibiotic susceptibility profile along with their MIC of all the *Staphylococcus aureus* isolates from the patients with IE is shown in Fig. 1. It has been observed that majority of the isolates were resistant to oxacillin (MRSA), cotrimoxazole, ciprofloxacin, levofloxacin and penicillin. None of the isolates were found resistant to vancomycin, teicoplanin, daptomycin and linezolid, although their minimum inhibitory concentrations were variable. Approximately 10% of the isolates were resistant to clindamycin. None of the isolates were found susceptible to penicillin with an MIC of $\geq$0.5 μg/ml (Fig. 2).

The antibiotic susceptibility pattern of *Pseudomonas aeruginosa* isolated from IE patients showed that the meropenem and amikacin were the only drugs with 100% susceptibility, though MIC of the isolates was variable. Gentamicin, cetazidime, and piperacillin-tazobactum were the next most active agent with a susceptibility of 75% followed by cefepime cefperazone + sulbactum, ciprofloxacin, levofloxacin and ticarcillin-clavulinic acid (Fig. 2).

Enterococcal isolates were found to be 100% susceptible to linezolid and daptomycin. Vancomycin and teicoplanin felled next in line of most active agent having a susceptibility of 75%, followed by gentamicin and clindamycin. Nonetheless, Enterococci were least susceptible to ciprofloxacin, levofloxacin, erythromycin, penicillin and tetracycline with a susceptibility of only 25% (Fig. 3).

Both the *Escherichia coli* isolates obtained in the present study were found susceptible to all the drugs tested except cefuroxime and ciprofloxacin. One of the *E. coli* isolate was found resistant to cefipime and cotrimoxazole as well. On the contrary one of the two *Klebsiella pneumonaie* isolate of the present study was found to be resistant to all the antimicrobials tested as per CLSI recommendations except imipenam which also had reduced susceptibility. The antibiotic susceptibility pattern for *Stenotrophomoas maltophila* and *Achromobacter xylosoxidans* could not be evaluated as the CLSI breakpoints has not been defined for these organisms.

Out of the three fungal isolates obtained, 2 were identified as *Candida famata* and the third one was identified as *Candida tropicalis*. Antifungal susceptibility profile of *C. famata* was evaluated using CLSI breakpoints for Candida species, because the breakpoints for *C. famata* were not defined in CLSI guidelines. On the other hand the breakpoints for *C. tropicalis* were available and their susceptibility was evaluated accordingly. All the three isolates were susceptible to fluconazole, voriconazole, amphotericin B, caspofungin, micafungin and flucytocine.

A comparison was made between the antimicrobial susceptibility profile of the predominant organisms (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) isolated from IDU and Non IDU IE

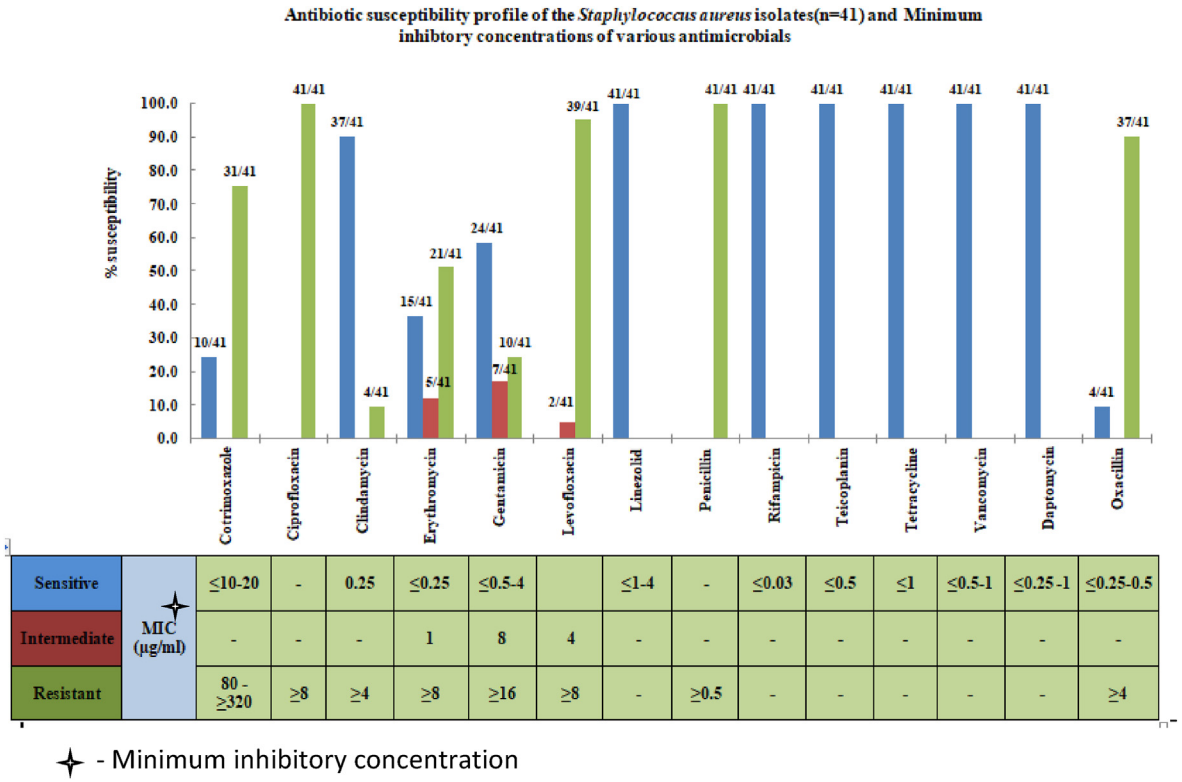

Fig. 1. Antibiotic susceptibility profile of the *Staphylococcus aureus* isolates (n = 41) and Minimum inhibtory concentrations of various antimicrobials.

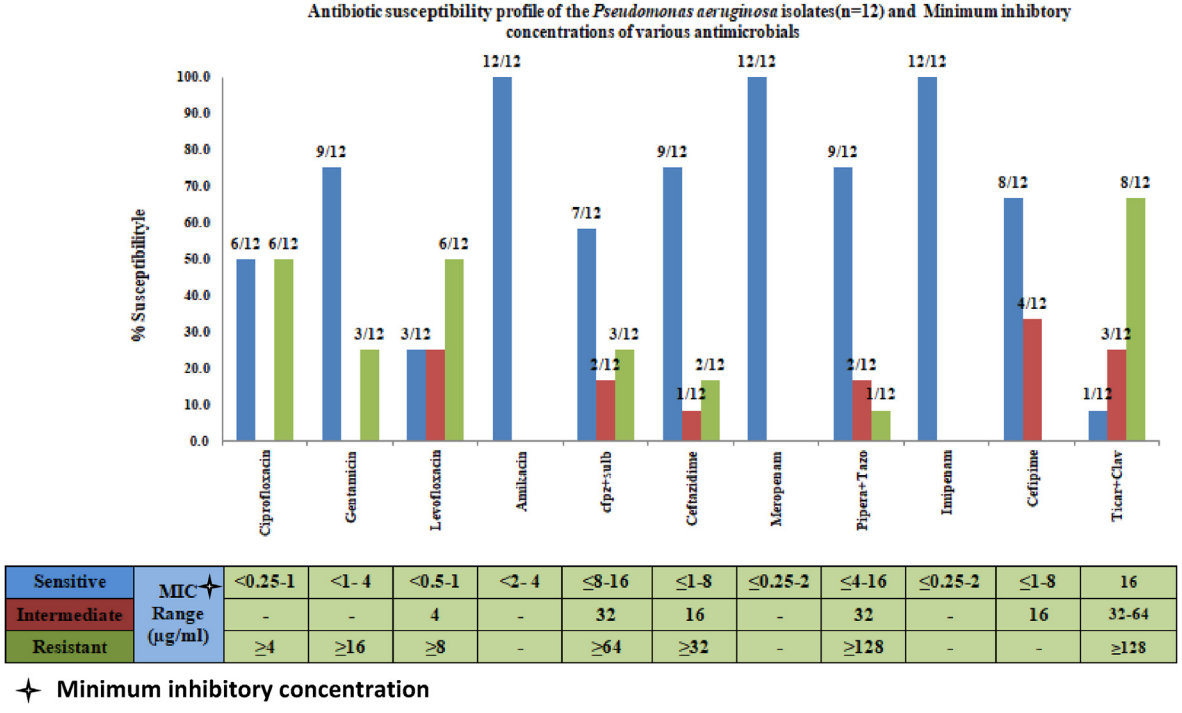

Fig. 2. Antibiotic susceptibility profile of the *Pseudomonas aeruginosa* isolates (n = 12) and Minimum inhibtory concentrations of various antimicrobials.

patients, against the drugs used for the treatment, as per ESC/AHA recommendations. However no statistically significant difference ($p > 0.01$) was observed in the susceptibility of these drugs between the two groups (Tables 1 and 2).

4. Discussion

On the basis of Duke's criteria[10] and several subsequent evidence based studies positive blood culture (at least 2 positive

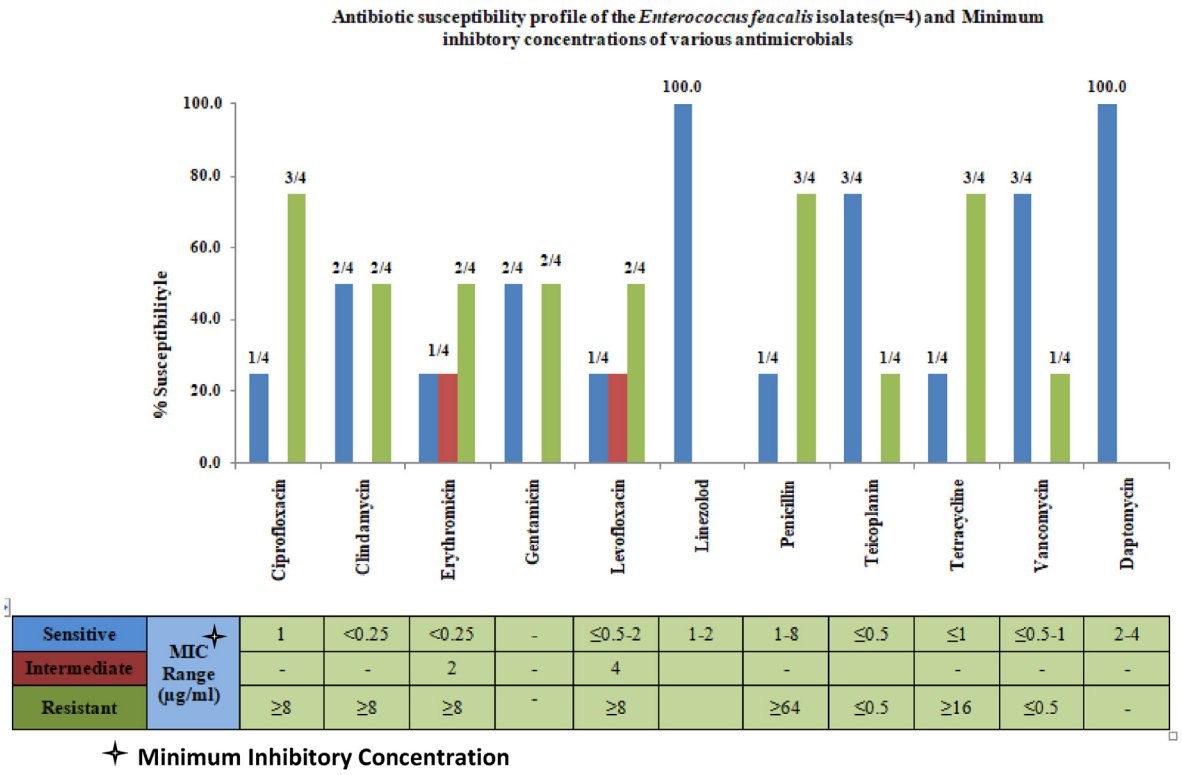

Fig. 3. Antibiotic susceptibility profile of the *Enterococcus fecalis* isolates (n = 4) and Minimum inhibtory concentrations of various antimicrobials.

cultures of blood samples drawn >12 h apart or all 3 or a majority of ≥4 separate cultures of blood, with first and last sample drawn at least 1 h apart) has been considered as one of the major criteria for the definite diagnosis of IE.[2,10,12]

The present study documents 49.9% positivity of blood culture in patient with IE. This finding is in rationale with many other studies from developing countries.[13–15] On the contrary, many other studies from different countries have doucumented higher but variable blood culture positivity in patients with IE.[16,17] However a significant association between previous intake of antibiotics and culture negative endocarditis has been reported in various studies[14,18] In the present study, low blood culture positivity may be attributed to indiscriminate use of antibiotics prior to hospitalization and non availability of investigations for rare and fastidious organisms like *Coxiella burnetti*, Bartonella, Brucella and HACEK group of organisms.

The present study highlights few other trends in the microbiological profile of patients with infective endocarditis. A major change has been observed in the distribution of etiological agent responsible for infective endocarditis as compared to the previous studies,[15,20] specifically the emergence of *Staphylococcus aureus* as the leading cause of infective endocarditis followed by *Pseudomans aeruginosa and* Enteroccci. *Streptococcus viridians* (Oral streptococci) was not found to be associated with infective endocarditis in any of the patients included in the study. Absence of oral streptococci can be attributed to the use of broad spectrum antibiotics prior to any dental procedure.[19] However, few other studies carried out recently has confirmed Staphylococci as the predominant pathogen isolated from patients with infective endocarditis.[21,22] In the present study *S aureus* remained the predominant pathogen in the intravenous drug abusers (IDU) IE cases, however an observable increase in the cases of IE due to *Pseudomonas aeruginosa* has been observed in this group. which is in contrast to the previous observation, whereby Gram negative bacteremia was observed in only 1.3% of the IDU-IE cases.[23] However few studies in the past has documented *Pseudomans aeruginosa* as the leading cause of infective endocarditis specifically in IDU. The reason for this change may be attributed to the changing patient profile in terms of demographics, social and economic trends.[24,25]

As per American Heart association guidelines, endorsed by infectious disease society of America (2015) the drugs of choice for endocarditis caused by MSSA are the semi synthetic penicillinase-resistant penicillins,.naficillin or oxacillin. If the strain is shown to be penicillin susceptible, a first-generation cephalosporin

Table 1
Comparison of Antimicrobial susceptibility profile (Number/%age) of *Staphylococcus aureus* ($n = 41$) isolates from IDU[a]/Non IDU patients with IE[b], against the recommended drugs for the treatment of IE.

	Oxacillin	Cotrimoxazole	Clindamycin	Vancomycin	Daptomycin
IDU (n = 26)	3/11.5	7/26.9	3/11.5	26/100	26/100
NON IDU (n = 15)	1/6.7	3/20	1/6.7	15/100	15/100
Total (n = 41)	4/9.8	10/24	4/9.8	41/100	41/100
p-value (λ[b])	0.612	0.619	0.612	1.00	1.00

[a] Intravenous drug users.
[b] Infective endocarditis.

Table 2
Comparison of Antimicrobial susceptibility profile (Number/%age) of Pseudomans aeruginosa ($n = 12$) isolates from IDU[a]/Non IDU patient with IE[b], against the recommended drugs for the treatment of IE.

	Piperacillin + tazobactum	Ticarcillin + clavulanate	Imipenam	Meropenam	Amikacin	Gentamicin	Ciprofloxacin	Levofloxacin	Ceftazidime	Cefipime
IDU (n = 10)	8/80	1/10	10/100	10/100	10/100	8/80	5/50	3/30	8/80	7/70
NON IDU (n = 2)	1/50	0/0	2/100	2/100	2/100	1/50	1/50	0/0	1/50	1/50
Total (n = 12)	9/75	1/8.3	12/100	12/100	12/100	9/75	6/50	3/25	9/75	8/66.7
P value (λ[b])	0.371	0.64	1	1	1	0.371	1	0.545	0.317	0.584

[a] Intravenous drug users.
[b] Infective endocarditis.

(cefazolin) is an effective alternative, specifically for patients with a history of nonanaphylactoid type penicillin allergy[2] However relapse of type A ß-lactamase-producing *Staphylococcus aureus* native valve endocarditis during cefazolin therapy has also been reported which raised a serious concern over the use of the drug.[26] Nonetheless, as per AHA guidelines cefazolin should not be used in patients with anaphylactoid type hypersensitivity to β-lactam drugs.[5] In the present study, however none of the MSSA were found susceptible to penicillin with an MIC of >0.5 μg/ml. Therefore, the use cefazolin in this category of patients needs to be reconsidered. Further, European Society of Cardiology (ESC) guidelines recommends the use of cotrimoxazole with clindamycin, as an alternative therapy for the treatment of infective endocarditis due to MSSA.[27] In the present study majority of the MSSA were found resistant to cotrimoxazole with an MIC of ≥320 μg/ml. Therefore the use of cotrimoxazole in combination with clindamycin may lead to clinical failure. To conclude, vancomycin remains the drug of choice for patients of endocarditis due to MSSA with life-threatening penicillin allergy. However, few studies have documented substantial clinical failure with vancomycin, in patients with *MSSA* endocarditis.[28,29] Hence alternative drugs or regimens needed to be worked out for the treatment of IE caused by MSSA. Hughes et al evaluated the continuous infusion of oxacillin as an effective alternative to intermittent infusion of oxacillin for the treatment of infective endocarditis due to MSSA. Continuous infusion regimen of oxacillin had better microbiological cure with ease of administration and pharmacodynamic optimization, hence deserves attention.[30]

Vancomycin remains the therapeutic choice for the treatment of serious infections including infective endocarditis due to MRSA and so are the recommendation of AHA[5] and ECS.[27] A prospective study in patients with *Staphylococcus aureus* serious infections including IE, showed that vancomycin AUC/MIC ratios of >421 was associated with the better patient outcome and this ratio is well achievable with trough serum concentration 15 mg/l and recommended dose of 30 mg/kg per 24 h IV in 2 equally divided doses, if vancomycin MIC was <1 μg/ml.[31] In the current study all the Staphylococcal isolates had MIC of vancomycin varying between ≤0 0.5 -1 μg/ml. Few studies have also evaluated the efficacy of combination therapy (ciprofloxacin-rifampicin and methicillin/gentamicin) for the treatment of IE in IDUs and reported a cure rate of 90%.[32,33] But, all the Staphylococcal isolates in this study were found to be resistant to ciprofloxacin with very high MIC of >8 μg/ml, though rifampicin was found to be 100% effective against these isolates.

With the changing demographics, clinical and microbiological profile of the endocarditis *Pseudomonas aeruginosa* has been increasingly reported to be associated with infective endocarditis specifically in intravenous drug abusers. Its antibiotic susceptibility profile deserves attention as wide variations have been reported in the resistance pattern of this organism depending upon the geographic location within the country and across the country as well.[34–39] However, most of the available literature on drug resistance in *P aeruginosa* isolates is based upon clinical isolates obtained from different clinical samples with variable clinical presentation and showed inconsistent susceptibility profile. The present study laid emphasizes on the antibiotic susceptibility along with the MIC (μg/ml) of the various drugs tested against *P. aeruginosa* isolated from patients with infective endocarditis.

The therapeutic option recommended by AHA for the treatment of IE due to non HACEK gram negative organisms specifically *Pseudomonas aeruginosa* is cardiac surgery followed by prolonged courses of antibiotic therapy. Combination antimicrobial therapy includes a β-lactam (penicillins, cephalosporins, or carbapenems) and either an aminoglycoside or a fluoroquinolone. ESC also recommends the use of cotrimoxazole if required. However CLSI did not recommend the testing of cotrimoxazole against *Pseudomonas aeruginosa*. All the *Pseudomonas aeruginosa* isolates of the present study were susceptible to carbapenems (Imipenam and meropenam); These isolates were also tested against the anti pseudomonas penicllins with β-lactamase inhibitor combination as per CLSI recommendations. A very high level (91.7%) of resistance was observed against ticarcillin-clavulanate combination with many of the isolates having an MIC >128 μg/ml. However, only 25% of the isolates showed decreased susceptibility/resistance to piperacillin-tazobactum combination Similarly, anti pseudomonas cephalosporins and cephalosporin- β lactamase inhibitors combination showed significant resistance against *Pseudomonas aeruginosa* isolates in the present study. In contrast all the *P. aeruginosa* isolates were susceptible to amikacin (aminoglycosides), but gentamicin is not as effective with some of the isolates having an MIC as high as ≥16 μg/ml. Further for concentration-dependent antibiotics such as amikacin/gentamicin and fluoroquinolones like ciprofloxacin and levofloxacin a ratio of maximum serum concentration to MIC of >10 was associated with improved efficacy in patients with Gram-negative serious infections, whereas ratio of the area under the 24-h plasma concentration−time curve to the MIC (AUC24/MIC) > 125 was associated with an improved clinical efficacy for ciprofloxacin against infections caused by *Pseudomonas aeruginosa.*[40] As the present study witnessed a very high MICs of these drugs (except amikacin) the desired effective concentrations to have in-vivo efficacy may not be achievable with the standard regimens. Therefore local antibiogram of the isolates has to be kept in mind while treating the patient as per AHA/ECS recommendation,[11] specifically in IDU, as 83.3% of the *Pseudomonas aeruginosa* isolates of the present study were obtained from IDU-IE patients.

The number of gram negative enterobactericae, Enterococci and fungal isolates obtained from the patients with IE in the present study were too less to discuss their antimicrobial susceptibility profile in relation to AHA/ESC guidelines and a validated treatment recommendation is difficult to determine. Further clinical studies are warranted in this direction.

5. Conclusion

To conclude, the study provide a clear evidence of change in demographic profile of the patients with predominance of intravenous drug abusers among the patients diagnosed with infective endocarditis. In addition there is a change observed in the microbiological profile in terms of distribution of the causative agents and their antimicrobial susceptibility profile. However, no statistically significant difference in the AST pattern of isolates from IDU vs. non IDU patients was seen. Therefore local antibiograms of the causative agents should be kept in mind while following AHA/ESC regimens. (Tables 1 and 2, Fig. 3)

Limitation of the study

The number of gram negative isolates (enterobactericae), Enterococci and fungal isolates obtained from the patients with IE in the present study were too less to discuss their antimicrobial susceptibility profile in relation to AHA/ESC guidelines. Further evidence based clinical data based upon the treatment given and its outcome needs to be analyzed.

Ethical clearance

The study has been approved by institutional ethical committee (DMCH/P/2018/32 dated 11.01.2018).

Declaration of competing interest

All authors have none to declare.

References

1. Cahill TJ, Baddour LM, Habib G, et al. Challenges in infective endocarditis. *J Am Coll Cardiol*. 2017;69(3):325–344. https://doi.org/10.1016/j.jacc.2016.10.066.
2. Baddour LM, Wilson WR, Bayer AS, et al. Infective endocarditis in adults: diagnosis, antimicrobial therapy, and management of complications: a scientific statement for healthcare professionals from the American Heart Association. *Circulation*. 2015;132(15):1435–1486. https://doi.org/10.1161/CIR.0000000000000296.
3. Osler W. The Gulstonian lectures on malignant endocarditis. *BMJ*. 1885;1:467–470. https://doi.org/10.1136/bmj.1.1262.467, 522-526, 577-9.
4. Slipczuk L, Codolosa JN, Davila CD, et al. Infective endocarditis epidemiology over five decades: a systematic review. *PLoS One*. 2013;8(12), e82665. https://doi.org/10.1371/journal.pone.0082665.
5. Goyal A, Mohan B, Kumar P, et al. Clinical characteristics and outcome of infective endocarditis among intravenous drug abusers in India. *Indian Heart J*. 2020;72(6):547–551. https://doi.org/10.1016/j.ihj.2020.09.014.
6. Federspiel JJ, Stearns SC, Peppercorn AF, Chu VH, Fowler VG. Increasing US rates of endocarditis with Staphylococcus aureus: 1999-2008. *Arch Intern Med*. 2012;172(4):363–365. https://doi.org/10.1001/archinternmed.2011.1027.
7. Devlin RK, Andrews MM, Von Reyn CF. Recent trends in infective endocarditis: influence of case definitions. *Curr Opin Cardiol*. 2004;19:134–139. https://doi.org/10.1097/00001573-200403000-00012.
8. Cecchi E, Forno D, Imazio M, et al. New trends in the epidemiological and clinical features of infective endocarditis: results of a multicenter prospective study. *Ital Heart J*. 2004;5:249–256.
9. Durack DT, Lukes AS, Bright DK, Service DE. New criteria for diagnosis of infective endocarditis: utilization of specific echocardiographic findings. *Am J Med*. 1994;96(3):200–209. https://doi.org/10.1016/0002-9343(94)90143-0.
10. Li JS, Sexton DJ, Mick N, et al. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. *Clin Infect Dis*. 2000;30(4):633–638. https://doi.org/10.1086/313753.
11. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing*. CLSI supplement M100; 2017.
12. Berlin JA, Abrutyn E, Strom BL, et al. Assessing diagnostic criteria for active infective endocarditis. *Am J Cardiol*. 1994;73(12):887–891. https://doi.org/10.1016/0002-9149(94)90816-8.
13. Tariq M, Alam M, Munir G, Khan MA, Smego Jr RA. Infective endocarditis: a five-year experience at a tertiary care hospital in Pakistan. *Int J Infect Dis*. 2004;8(3):163–170. https://doi.org/10.1016/j.ijid.2004.02.001.
14. Khan NU, Farman MT, Sial JA, Achakzai AS, Saghir T, Ishaq M. Changing trends of infective endocarditis. *J Pakistan Med Assoc*. 2010;60(1):24–27.
15. Agarwal R, Bahl VK, Malaviya AN. Changing spectrum of clinical and laboratory profile of infective endocarditis. *J Assoc Phys India*. 1992;40:721–723.
16. Romano G, Carozza A, Della Corte A, et al. Native versus primary prosthetic valve endocarditis: comparison of clinical features and long-term outcome in 353 patients. *J Heart Valve Dis*. 2004;13(2):200–209.
17. Nakatani S, Mitsutake K, Hozumi T, et al. Current characteristics of infective endocarditis in Japan: an analysis of 848 cases in 2000 and 2001. *Circ J*. 2003;67(11):901–905. https://doi.org/10.1253/circj.67.901.
18. Hogevik H, Olaison L, Andersson R, Lindberg J, Alestig K. Epidemiologic aspects of infective endocarditis in an urban population: a 5-year prospective study. *Medicine (Baltim)*. 1995;74:324–339. https://doi.org/10.1097/00005792-199511000-00003.
19. Chen PC, Tung YC, Wu PW, et al. Dental procedures and the risk of infective endocarditis. *Medicine*. 2015;94(43), e1826. https://doi.org/10.1097/MD.0000000000001826.
20. Hoen B, Alla F, Selton-Suty C, et al. Changing profile of infective endocarditis: results of a 1-year survey in France. *Jama*. 2002 Jul 3;288(1):75–81. https://doi.org/10.1001/jama.288.1.75.
21. Selton-Suty C, Célard M, Le Moing V, et al. Preeminence of *Staphylococcus aureus* in infective endocarditis: a 1-year population-based survey. *Clin Infect Dis*. 2012;54(9):1230–1239. https://doi.org/10.1093/cid/cis199.
22. Mohiyiddeen G, Brett I, Jude E. Infective endocarditis caused by Staphylococcus aureus in a patient with atopic dermatitis: a case report. *J Med Case Rep*. 2008;2:143. https://doi.org/10.1186/1752-1947-2-143. doi: 10.1186/1752-1947-2-143.
23. Kadri AN, Wilner B, Hernandez AV, et al. Geographic trends, patient characteristics, and outcomes of infective endocarditis associated with drug abuse in the United States from 2002 to 2016. *Journal of the American Heart Association*. 2019;8(19), e012969. https://doi.org/10.1161/jaha.119.012969.
24. Levin MH, Weinstein RA, Nathan C, Selander RK, Ochman H, Kabins SA. Association of infection caused by *Pseudomonas aeruginosa* serotype O11 with intravenous abuse of pentazocine mixed with tripelennamine. *J Clin Microbiol*. 1984;20(4):758–762. https://doi.org/10.1128/JCM.20.4.
25. Lin TI, Huang YF, Liu PY, et al. Pseudomonas aeruginosa infective endocarditis in patients who do not use intravenous drugs: analysis of risk factors and treatment outcomes. *J Microbiol Immunol Infect*. 2016;49(4):516–522. https://doi.org/10.1128/JCM.20.4.
26. Nannini EC, Singh KV, Murray BE. Relapse of type A ß-lactamase-producing *Staphylococcus aureus* native valve endocarditis during cefazolin therapy: revisting the issue. *Clin Infect Dis*. 2003;37:1194–1198. https://doi.org/10.1086/379021.
27. Habib G, Lancellotti P, Antunes MJ, et al, ESC Scientific Document Group. 2015 ESC guidelines for the management of infective endocarditis: the task force for the management of infective endocarditis of the European society of Cardiology (ESC). Endorsed by: European association for cardio-thoracic surgery (EACTS), the European association of nuclear medicine (EANM). *Eur Heart J*. 2015 Nov 21;36(44):3075–3128. https://doi.org/10.1093/eurheartj/ehv319.
28. Small PM, Chambers HF. Vancomycin for *Staphylococcus aureus* endocarditis in intravenous drug users. *Antimicrob Agents Chemother*. 1990;34:1227–1231. https://doi.org/10.1128/aac.34.6.1227.
29. Gentry CA, Rodvold KA, Novak RM, Hershow RC, Naderer OJ. Retrospective evaluation of therapies for Staphylococcus aureus endocarditis. *Pharmacotherapy*. 1997;17(5):990–997.
30. Hughes DW, Frei CR, Maxwell PR, et al. Continuous versus intermittent infusion of oxacillin for treatment of infective endocarditis caused by methicillin-susceptible Staphylococcus aureus. *Antimicrobial agents and chemotherapy*. 2009;53(5):2014–2019. https://doi.org/10.1128/AAC.01232-08.
31. Rybak M, Lomaestro B, Rotschafer JC, et al. Therapeutic monitoring of vancomycin in adult patients: a consensus review of the American society of health-system pharmacists, the infectious diseases society of America, and the society of infectious diseases pharmacists. *Am J Health Syst Pharm*. 2009;66(1):82–98. https://doi.org/10.2146/ajhp080434.
32. Dworkin RJ, Sande MA, Lee BL, Chambers HF. Treatment of right-sided Staphylococcus aureus endocarditis in intravenous drug users with ciprofloxacin and rifampicin. *Lancet*. 1989;334(8671):1071–1073. https://doi.org/10.1016/s0140-6736(89)91083-0.
33. Murray HW, Wigley FM, Mann JJ, Arthur RR. Combination antibiotic therapy in staphylococcal endocarditis: the use of methicillin sodium-gentamicin sulfate therapy. *Arch Intern Med*. 1976 Apr 1;136(4):480–483. https://doi.org/10.1001/archinte.1976.03630040082017.
34. Ka R, Diagne R, Niang AA, et al. Epidemiological aspects and antibiotic resistance profile of Pseudomonas aeruginosa in fann CHNU. *J Bacteriol Mycol*. 2020;7(1):1125.
35. Sader HS, Huband MD, Castanheira M, Flamm RK. Pseudomonas aeruginosa antimicrobial susceptibility results from four years (2012 to 2015) of the international network for optimal resistance monitoring program in the United States. *Antimicrobial agents and chemotherapy*. 2017;61(3). https://doi.org/10.1128/aac.02252-16. e02252-16.
36. Ghasemian A, Rizi KS, Vardanjani HR, Nojoomi F. Prevalence of clinically isolated metallo-beta-lactamase-producing Pseudomonas aeruginosa, coding genes, and possible risk factors in Iran. *Iranian J Pathol*. 2018;13(1):1–9.
37. Chandrasekaran K, Gandhiraj D, Mohanta GP, Rajasekaran A. Antibacterial susceptibility profile of Pseudomonas aeruginosa in a private hospital, India. *Intern J Res Pharmaceutical Sci*. 2019;10(1):298–302.
38. Javiya VA, Ghatak SB, Patel KR, Patel JA. Antibiotic susceptibility patterns of Pseudomonas aeruginosa at a tertiary care hospital in Gujarat, India. *Indian J*

Pharmacol. 2008;40(5):230. https://doi.org/10.4103/0253-7613.44156: 10.4103/0253-7613.44156.
39. Kaur S, Gupta V, Chhina D. AmpC β-lactamases producing Gram-negative clinical isolates from a tertiary care hospital. *J Mahatma Gandhi Inst Med*. 2016;21:107–110. https://doi.org/10.4103/0971-9903.189526. Sci.
40. Forrest AL, Nix DE, Ballow CH, Goss TF, Birmingham MC, Schentag JJ. Pharmacodynamics of intravenous ciprofloxacin in seriously ill patients. *Antimicrobial agents and chemotherapy*. 1993;37(5):1073–1081. https://doi.org/10.1128/aac.37.5.1073.