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

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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 ($\chi^2$ 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.

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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 narrative of infective endocarditis in the year 19853 and has been discussed extensively in many studies from industrialized world. The microbiological profile, associated risk factors and demographic characteristics of the patients with infective 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.6,7 Staphylococcus aureus and group D Streptococci (enterococci) has substantially

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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 intraintravenous drug abusers.7 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 Candida species (2 isolates of Candida famata and one isolate of Candida tropicalis) and one isolate each of Acromobacter xylosidans and Streptococcus agilactiae were obtained as has been listed in the previous study.

Antibiotic susceptibility pattern of these isolates was analyzed. The isolates were labeled as sensitive, intermediate 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), cefotaxime, ciprofloxacin, levofoxacin 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 <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, cefazidime, and piperacillin-tazobactum were the next most active agent with a susceptibility of 75% followed by cefepime cefepime+ sulbactum, ciprofloxacin, levofloxacin and ticaricillin-clavulanic 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 cefepime and cotrimoxazole as well. On the contrary one of the two Klebsiella pneumoniae 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 Stenotrophomomas maltophilia and Acromobacter xylosidans 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 fluconazole.

A comparison was made between the antimicrobrial susceptibility profile of the predominant organisms (Staphylococcus aureus and Pseudomonas aeruginosa) isolated from IDU and Non IDU IE patients and the antibiotic susceptibility pattern for these isolates obtained. Chi square 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.
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' and several subsequent evidence based studies positive blood culture (at least 2 positive
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 documented 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 studies14,18. 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 Pseudomonas 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 Pseudomonas 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,.na If the strain is shown to be penicillin susceptible, a first-generation cephalosporin

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**Table 1**

Comparison of Antimicrobial susceptibility profile (Number/%age) of Staphylococcus aureus (n = 41) isolates from IDU/Non IDU patients with IEc, 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 (X²) | 0.612     | 0.619         | 0.612       | 1.00       | 1.00       |

a Intravenous drug users.

b Infective endocarditis.
(cefaclin) is an effective alternative, specifically for patients with a history of nonanaphylactoid type penicillin allergy. However, relapse of type A β-lactam-producing Staphylococcus aureus native valve endocarditis during cefazolin therapy has also been reported which raised a serious concern over the use of the drug. Nonetheless, as per AHA guidelines cefazolin should not be used in patients with anaphylactoid type hypersensitivity to β-lactam drugs. 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 recommend the use of ceftriaxone with clindamycin, as an alternative therapy for the treatment of infective endocarditis due to MSSA.

In the present study majority of the MSSA were found resistant to ceftriaxone with an MIC of ≥320 μg/ml. Therefore the use of ceftriaxone 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. 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.

Vancomycin remains the therapeutic choice for the treatment of serious infections including infective endocarditis due to MRSA and so are the recommendation of AHA and ESC. 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. In the current study all the Staphylococcal isolates had MIC of vancomycin varying between <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%. 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. 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 cefotaxime if required. However CLSI did not recommend the testing of cefotaxime against Pseudomonas aeruginosa. All the Pseudomonas aeruginosa isolates of the present study were susceptible to carbapenems (imipenem and meropenem); These isolates were also tested against the anti pseudomonas penicillins 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. 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/ESC recommendations, 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 enterobacteriace, 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.

### Table 2

| Piperacillin + tazobactum | Ticarcillin + clavulanate | Imipenem | Meropenem | Amikacin | Gentamicin | Ciprofloxacin | Levofloxacin | Cefazidime | Cefepine |
|--------------------------|--------------------------|----------|-----------|----------|-----------|--------------|-------------|------------|----------|
| IDU (n = 10)             | 8/80                     | 1/10     | 2/100     | 2/100    | 1/50      | 1/50         | 3/30        | 3/30       | 8/80     |
| NON IDU (n = 2)          | 1/50                     | 0/0      | 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   | 9/75      | 6/50         | 3/25        | 9/75       | 8/66.7   |
| P value (k)              | 0.371                    | 0.64     | 1         | 1        | 1         | 0.371        | 1           | 0.545      | 0.317    | 0.584    |

* Intravenous drug users.

** Infective endocarditis.
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 (enterobacteriae), 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.

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