BACTERIAL ISOLATION AND ANTIMICROBIAL SUSCEPTIBILITIES IN PATIENTS WITH INFECTIVE ENDOCARDITIS

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Abstract. Infective endocarditis is life-threatening and urgent treatment is needed. We review here 6,217 patients with infective endocarditis admitted to Siriraj Hospital during a 20 year period, 1982-2001. In this retrospective study, we obtained our data from hemoculture results. Bacterial pathogens were found in only 834 patients (13.41%). Of the five most common pathogens, nonfermentative gram-negative rods (N.F.) ranked first (20.94%), followed by coagulase-negative staphylococci (12.47%), viridans streptococci (10.23%), *Staphylococcus aureus* (9.29%), and *Escherichia coli* (8.59%). Of the N.F., the sensitivities were: cefoperazone/sulbactam (86%), piperacillin/tazobactam (86%), meropenem (84%), imipenem (83%), ceftazidime (78%), ofloxacin (85%), ciprofloxacin (71%), and co-trimoxazole (71%).

INTRODUCTION

Infective endocarditis, a microbial infection of the endocardial surface of the heart, is uncommon, but can cause significant morbidity and mortality. Endocarditis usually develops in patients with structural cardiac defects who develop bacteremia with organisms likely to cause endocarditis. Bacteremia may occur spontaneously, or as a result of a focal infection, such as a urinary tract infection, pneumonia or cellulitis. The diagnosis of endocarditis is usually made in febrile patients with persistent bacteremia and the presence of vegetations on echocardiography or on gross examination or histologic evaluation of a removed heart valve.

Organisms which commonly cause infective endocarditis are gram-positive cocci: viridans streptococci, coagulase-negative staphylococci, *Corynebacterium* species, enterococci, *Streptococcus bovis* and microorganisms in the HACEK group, namely *Haemophilus parainfluenzae, H. influenzae, H. aphrophilus, Actinobacillus actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens* and *Kingella kingae* (Wilson *et al*, 1995). Recently, the epidemiology of infective endocarditis in developed countries has been changing as a result of longevity, new predisposing factors and an increase in nosocomial cases (Mylonakis and Calderwood, 2001). Other conditions associated with an increased incidence of infective endocarditis included poor dental hygiene, long term hemodialysis and diabetes mellitus (Strom *et al*, 2000). The purpose of this study was to analyze the data of bacterial pathogens isolated from the hemocultures of patients admitted to Siriraj Hospital with the clinical diagnosis of infective endocarditis over a 20 year period (1982-2001).

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MATERIALS AND METHODS

In this retrospective study, the data was obtained from hemoculture results. There were 6,217 patients admitted to Siriraj Hospital with the clinical diagnosis of infective endocarditis. Hemocultures were performed by inoculating blood from each patient into conventional hemoculture broth using a ratio of 1:5 to 1:10, or Bactec-automated bottles, as requested by clinicians, and incubated at 35ºC. For conventional hemoculture, after 6 hours of incubation, most bacteria responsible for clinically significant infections were present in numbers large enough to recover by blind subculture. Culture negative bottles were then reincubated for 14 days. Each bottle was examined daily for evidence of growth, indicated by turbidity, hemoly-

sis or the presence of small colonies. If this was present, an identification was performed by aseptically removing a few drops of the well-mixed hemoculture broth and spreading this onto blood agar. This was then incubated in 5% CO_2 at 35ºC for 1-2 days. Bacterial pathogens were isolated and identified according to standard microbiological techniques (Murray *et al*, 2003).

Antimicrobial susceptibility testing

The Kirby-Bauer disk diffusion method was performed according to the National Committee for Clinical Laboratory Standards (NCCLS, 2004). The Muller-Hinton plates were incubated at 35ºC for 18-24 hours, after which the diameter of each inhibition zone was measured.

RESULTS

Our study demonstrated that among 6,217 hemoculture results, only 834 (13.41%) were positive for bacterial pathogens (Table 1). Of the five most common pathogens, non fermentative gram-negative rods ranked first (20.94%), followed by coagulase-negative staphylococci (12.47%), viridans streptococci (10.23%), *Staphylococcus aureus* (9.29%), and *Escherichia coli* (8.59%). Other bacteria, including *Klebsiella pneumoniae* (6.47%), *Salmonella* serogroups A, B, C, D, and E (4.58%), *Acinetobacter baumannii* (4.47%), and enterococci (3.89%) were also isolated (Table 2).

Antimicrobial susceptibility testing

Nonfermentative gram-negative rods (N.F.). The percentages of N.F. susceptible to each drug are shown in Table 3. They were sensitive to cefoperazone/sulbactam (86%), piparacillin/tazobactam (86%), meropenem (84%), imipenem (83%), ceftazidime (78%), ofloxacin (85%), ciprofloxacin (71%), and co-trimoxazole (71%).

Table 1
Number of infective endocarditis patients and percentages of positive cultures for bacterial pathogens.

Infective endocarditis (IE) patients	Number of patients
Hemocultures in IE patients	6,217
Hemocultures positive for bacterial pathogens	834
% Positive	13.41

Table 2
Different bacterial pathogens isolated from infective endocarditis patients admitted to Siriraj Hospital, 1982-2001.

Bacterial pathogens	% Positive
1. Nonfermentative gram-negative rods	20.94
2. Coagulase-negative staphylococci	12.47
3. Viridans streptococci	10.23
4. *Staphylococcus aureus*	9.29
5. *Escherichia coli*	8.59
6. *Klebsiella pneumoniae*	6.47
7. *Salmonella* serogroups A, B, C, D, E	4.58
8. *Acinetobacter baumannii*	4.47
9. Enterococci	3.89

Table 3
Percentages of sensitivities for nonfermentative gram-negative rods.

Antimicrobial agents	% sensitivities of nonfermentative gram-negative rods
Ampicillin	28
Cefazolin	11
Cefotaxime	39
Ceftriaxone	38
Ceftazidime	78
Cefepime	56
Cefpirome	52
Ceftibuten	24
Amoxycillin/clavulanate	56
Ampicillin/sulbactam	55
Cefoperazone/sulbactam	86
Piperacillin/tazobactam	86
Imipenem	83
Meropenem	84
Gentamicin	49
Amikacin	51
Netilmycin	56
Ofloxacin	85
Ciprofloxacin	71
Co-trimoxazole	71

Coagulase-negative staphylococci. The coagulase-negative staphylococci were sensitive to vancomycin (100%), teicoplanin (96%), netilmycin (99%), amikacin (84%), cefoperazone/sulbactam (87%), piperacillin/tazobactam (86%), cefpirome (85%), cefazolin (85%), amoxycillin/clavulanate (82%), ampicillin/sulbactam (80%),

Table 4
Percentages of sensitivities for coagulase-negative staphylococci.

Antimicrobial agents	% sensitivities of coagulase-negative staphylococci
Ampicillin	19
Oxacillin	33
Cefazolin	85
Cefotaxime	53
Ceftriaxone	45
Ceftazidime	41
Cefepime	71
Cefpirome	85
Amoxycillin/clavulanate	82
Ampicillin/sulbactam	80
Cefoperazone/sulbactam	87
Piperacillin/tazobactam	86
Imipenem	79
Meropenem	72
Gentamicin	52
Amikacin	84
Netilmycin	99
Ofloxacin	73
Erythromycin	38
Vancomycin	100
Teicoplanin	96
Co-trimoxazole	54
Fosfomycin	39
Fusidic acid	76

imipenem (79%), fusidic acid (76%), meropenem (72%), cefepime (71%), and ofloxacin (73%) (Table 4).

Viridans streptococci. All viridans streptococci were 100% susceptible to vancomycin, teicoplanin, piperacillin/tazobactam, imipenem, meropenem, cefepime, cefpirome. They were sensitive to amoxycillin/clavulanate (95%), cefoperazone/sulbactam (92%), cefazolin (91%), ampicillin/sulbactam (86%), cefotaxime (84%), ceftriaxone (84%), cetazidime (79%), and ampicillin (72%). The susceptibility patterns for viridans streptococci are shown in Table 5.

Staphylococcus aureus. The percentages of *S. aureus* susceptible to each drug are shown in Table 6. They were sensitive to vancomycin (100%), teicoplanin (100%), fusidic acid (94%), fosfomycin (71%), co-trimoxazole (70%), and netilmycin (71%). There were methicillin-sensitive *S. aureus* (59%) and methicillin-resistant S. aureus (41%).

Table 5
Percentages of sensitivities for viridans streptococci.

Antimicrobial agents	% sensitivities of viridans streptococci
Penicillin	60
Ampicillin	72
Cefazolin	91
Cefotaxime	84
Ceftriaxone	84
Ceftazidime	79
Cefepime	100
Cefpirome	100
Amoxycillin/clavulanate	95
Ampicillin/sulbactam	86
Cefoperazone/sulbactam	92
Piperacillin/tazobactam	100
Imipenem	100
Meropenem	100
Gentamicin	39
Amikacin	7
Netilmycin	76
Ofloxacin	62
Ciprofloxacin	38
Erythromycin	56
Vancomycin	100
Teicoplanin	100
Co-trimoxazole	46

Escherichia coli. *E. coli* was sensitive to imipenem (100%), meropemen (100%), amikacin (97%), netilmycin (95%), gentamicin (72%), piperacillin/tazobactam (94%), cefepime (92%), and cetazidime (90%). The susceptibility patterns are shown in Table 7.

DISCUSSION

Endocarditis is a serious disease. Although advances in antimicrobial therapy and development of better diagnostic methods have reduced the morbidity and mortality of infective endocarditis, it remains a potentially life-threatening disease. A positive hemoculture is a major diagnostic criterion for infective endocarditis. Persistent bacteremia and a high frequency of positive hemocultures are typical of this infection. In this study, hemocultures were positive for bacterial pathogens in only 834 (13.41%) of 6,217 cases. Interestingly, the most common etiologic

Table 6
Percentages of sensitivities for *Staphylococcus aureus.*

Antimicrobial agents	% sensitivities of *S. aureus*
Ampicillin	10
Oxacillin	59
Cefazolin	60
Cefotaxime	59
Ceftriaxone	59
Ceftazidime	44
Cefepime	59
Cefpirome	71
Amoxycillin/clavulanate	61
Ampicillin/sulbactam	58
Cefoperazone/sulbactam	61
Piperacillin/tazobactam	62
Imipenem	67
Meropenem	60
Gentamicin	60
Amikacin	61
Netilmycin	71
Ofloxacin	60
Erythromycin	57
Vancomycin	100
Teicoplanin	100
Co-trimoxazole	70
Fosfomycin	71
Fusidic acid	94

agent isolated in this study was N.F., followed by coagulase-negative staphylococci, viridans streptococci, *S. aureus* and *E. coli*. These results differ from other studies (Petal *et al*, 2000). Staphylococci, particularly *S. aureus*, may be found in cases of acute infective endocarditis. Coagulase-negative staphylococci also cause infective endocarditis. One species of community-acquired coagulase-negative staphylococci, *S. lugdunensis*, is commonly associated with heart valve destruction and the requirement for valve replacement (Petal *et al*, 2000). The species of streptococci in patients with endocarditis continue to be *S. sanguis, S. bovis, S. mutans* and *S. mitis* (Mylonakis and Calderwood, 2001).

Observations from infective endocarditis patients in an Indian Hospital reported that hemocultures were positive in only 87 (47%) of 190 episodes of infective endocarditis. The commonest infecting pathogenic bacteria were staphylococci (37 cases) and streptococci (34 cases). Of the 103 culture negative patients, 87 (84%) received antimicrobial therapy before coming to the hospital. Staphylococcal infections are generally more fulminant and have a shorter duration of symptoms, whereas other infections tend to be slower in developing, thus, these patients are more likely to receive antimicrobial agents before cultures are taken (Choudhury *et al*, 1992).

Table 7
Percentages of sensitivities for *Escherichia coli.*

Antimicrobial agents	% sensitivities of *E. coli*
Ampicillin	18
Cefazolin	73
Cefotaxime	82
Ceftriaxone	81
Ceftazidime	90
Cefepime	92
Cefpirome	80
Ceftibuten	88
Amoxycillin/clavulanate	54
Ampicillin/sulbactam	46
Cefoperazone/sulbactam	89
Piperacillin/tazobactam	94
Imipenem	100
Meropenem	100
Gentamicin	72
Amikacin	97
Netilmycin	95
Ofloxacin	60
Ciprofloxacin	59
Co-trimoxazole	33

Endocarditis associated with open heart surgery is most often caused by *S. aureus*, coagulase-negative staphylococci or diphtheroids. No single antimicrobial regimen is effective against all these bacteria. Prophylaxis at the time of cardiac surgery should be directed primarily against staphylococci and should be of short duration. First generation cephalosporins are the most used, but the choice of antimicrobial agent(s) should be based on the antimicrobial susceptibility patterns of each hospital.

In our study, all coagulase-negative staphylococci, *S. aureus* and viridans streptococci were sensitive to vancomycin (100%). Endocarditis due to methicilin-resistant staphylococci (MRSA) should be treated with intravenous vancomycin. Treatment options for patients who cannot tolerate vancomycin are limited. Teicoplanin, a glycopeptide antimicrobial agent, has activity against

MRSA, and may be tolerated by some patients who are allergic to vancomycin (Gilbert *et al*, 1991; Bayer, 1993). Many strains of MRSA are also resistant to aminoglycosides. Evidence from experimental models of endocarditis due to MRSA and clinical experience in treating prosthetic valve endocarditis caused by MRSA suggested that the optimal antimicrobial therapy was vancomycin combined with rifampin and gentamicin. If coagulase-negative staphylococci were resistant to gentamicin, an aminoglycoside to which it was susceptible was substituted for gentamicin (Wilson *et al*, 1995).

For viridans streptococci, amoxicillin, ampicillin, and penicillin, were equally effective *in vitro*, however, amoxicillin was recommended because it was better absorbed from the gastrointestinal tract (Dajani *et al*, 1994). However, the treatment for all endocarditis cases is intravenous only. In our study, N.F. was the most common pathogen isolated from infective endocarditis patients. Prior reports showed that the gram-negative bacilli that usually cause endocarditis are from the HACEK group of bacteria. These pathogenic bacteria grow slowly in standard hemoculture media and recovery may require prolonged incubation. Although, other gram-negative bacilli bacteremia may occur following genitourinary and gastrointestinal tract surgery or with instrumentation, they were only rarely responsible for endocarditis (Dajani *et al*, 1997). Other bacteria were also isolated in our study, including *K. pneumoniae*, *Salmonella* serogroups A, B, C, D, and E, *A. baumannii* and enterococci. Both *K. pneumoniae* and *K. oxytoca* have been reported to cause endocarditis (Anderson and Janoff, 1998). *Klebsiella* endocarditis, including an antecedent *Klebsiella* urinary tract infection as a probable origin in 50% of patients, had a high degree of virulence, as evidenced by native-valve endocarditis and prosthetic-valve involvement in 60% and 30% of patients, respectively. When the diagnosis was delayed, death occurred in 49% of patients despite antimicrobial therapy with broad-spectrum cephalosporins and aminoglycosides (Brouqui and Raoult, 2001). *Salmonella* was a rare cause of infective endocarditis. *Salmonella* serotypes commonly known to cause endocarditis included *S. choleraesuis, S. typhimurium, S. enteritidis* and infrequenly *S. thompson* and *S. derby* serotypes. Cetriaxone is the drug of choice for salmonellosis (Khan *et al*, 2003).

In conclusion, our results suggest that the clinicians should be aware of N.F., which is a large group of bacteria. Their incidence and antimicrobial susceptibilities may provide guidelines for the selection of appropriate drugs for treatment.

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