Rapid Diagnosis of Community-Acquired Pneumonia Using the Bac T/ Alert 3D System

Malini R. Capoor, Deepthi Nair and Pushpa Aggarwal, B. Gupta

Department of Microbiology and Medicine, Vardhman Mahaveer Medical College & Safdarjung Hospital, New Delhi

We compared BacT/Alert 3D with conventional culture for the diagnosis of community-acquired pneumonia (CAP). Antimicrobial susceptibility testing of the isolates was performed with the disk diffusion method, and the minimum inhibitory concentration (MIC) was calculated. Automation was superior in terms of recovery and time to detect pathogens. The bacterial spectrum in CAP was *Streptococcus pneumoniae* (35.3%) *Staphylococcus aureus* (23.5%), *Klebsiella pneumoniae* (20.5%) and *Haemophilus influenzae* (8.8%). Three of the 12 *S. pneumoniae* isolates showed penicillin resistance on MIC and two showed erythromycin resistance. There were two *H. influenzae* strains resistant to penicillin; these were beta lactamase producers. One-fourth of the *S. aureus* were oxacillin resistant. All isolates were sensitive to cefepime by disc diffusion and MIC methods. In the treatment of CAP, cefotaxime and cefepime are useful drugs when given as empirical therapy against multidrug resistant strains. The use of automation is vital in CAP, as rapid diagnosis and effective therapy can reduce mortality.

Key Words*:* community-acquired pneumonia, *Streptococcus pneumoniae, cefepime*.

Septicemia is the commonest cause of morbidity and mortality in critically-ill patients. In septicemia, the primary focus is the respiratory or urinary tract [1]. Acute respiratory infections (ARI) are foremost among infectious killers according to the World Health Organization (WHO) [2]. They cause a million deaths in India annually, of which 10%-15% are due to community-acquired pneumonia (CAP). The case fatality rate of bacterial CAP is 50% higher than that of viral pneumonia, in spite of a high incidence of viral pneumonia. When the etiological agent of CAP is diagnosed early and treated aggressively, it is possible to achieve cure [3,4].

Diagnosis in CAP is established by blood, pleural fluid or sputum cultures. Sputum yield is <50%, and it is often contaminated by colonizers [5]. Although, the diagnostic yield of blood culture in bacteremic CAP is low (10%-30%), the mortality is high. Therefore, whenever blood culture (BC) is positive, empirical therapy should be initiated [6]. Emerging antimicrobial resistance in respiratory pathogens is a global concern [7].

Automated, continuous-monitoring, blood culture systems have been developed; they are labor and time saving, have a lower contamination risk, a reduced incubation period and a higher isolation rate than conventional systems. The BacT/ Alert 3D (Bio Merieux, France) is a fully automated colorimetric, blood culture system, which incubates and agitates cultures; it has membrane sensors for detecting microbial growth based on CO_2 and pH changes [8-10]. There are a limited number of studies that compare BacT / Alert 3D with conventional culture for the diagnosis of pneumonia, particularly in low-resource settings [11,12]. We made this study to make such a comparison in a hospital in India.

Received on 19 May 2006; revised 6 October 2006.

Address for correspondence: Dr. Dr Malini R Capoor. C-99, Opp. Sainik Vihar, Peetampura, New Delhi-34, India. E-mail: rajeevmalini@rediffmail.com.

The Brazilian Journal of Infectious Diseases 2006;10(5):352-356.

Material and Methods

Our study was conducted at the department of Microbiology, Vardhman Mahaveer Medical College, in Safdarjung Hospital, a 1,700 bed tertiary care center, for a period of 10 months (August 2002 to May 2003). One-hundred-twenty-four cases of CAP attended at the Medicine and Pediatric department were included in our study. A case was defined clinically as a patient with fever, purulent cough, pleuritic chest pain and signs of pulmonary consolidation on chest x-ray.

Blood, sputum or pleural fluid was collected prior to initiation of antibiotics. Equal aliquots of 5 mL blood were collected from cases and aseptically inoculated into BacT/ Alert and brain heart infusion broth (BHIB) bottles. These were incubated for five and seven days, respectively and were also subcultured. When the initial BHIB culture was positive, a Gram's smear was examined and broth from the vial was subcultured onto the appropriate media. Using the final subculture on solid media as the gold standard, false-positive cases were defined for both methods as those that tested positive, but were negative on Gram's stain and final subculture. Instrument-negative or visual-inspection negative BHIB cultures that gave growth on final subculture were categorized as false negatives [9]. The conventional blood culture, sputum and pleural fluid processing and identification of all the isolates were performed by standard microbiological procedures [1].

Antimicrobial susceptibility testing of the isolates was performed by the Kirby Bauer disc diffusion method, following NCCLS guidelines [13]. For Gram-positive isolates, the antimicrobials included amoxycillin / clavulanic acid (20/10 μg), ciprofloxacin (30 μg), cefepime (30 μg), cefotaxime (30

μg), erythromycin (15 μg), penicillin (10 U), vancomycin (30 μg), penicillin (10 U) and Oxacillin (1μg), where relevant. The minimum inhibitory concentration (MIC) to cefotaxime, erythromycin, penicillin and vancomycin was determined using E strips (AB Biodisk). Beta lactamase production was tested using a nitrocefin disc. Oxacillin resistance was confirmed by agar dilution MIC testing for *Staphylococcus aureus*. For Gram-negative isolates, the antimicrobials were amoxycillin/clavulanic acid (20/10 μg), ciprofloxacin (5 μg), cefepime (10 μg), cefotaxime (30 μg) and amikacin (30 μg). The screening test for extended-spectrum beta lactamase (ESBL) was done with the double disc diffusion method, using cefotaxime (30 μg), ceftriaxone (30 μg), cefoperazone (75 μg), and augmentin (20/10 μg) discs. The MIC for ciprofloxacin, cefepime, cefotaxime and amikacin was determined by the agar dilution method.

Results

Patient population and clinical data

One-hundred-twenty-four CAP patients were included in the study. The median age was four years for pediatric patients and 48 years for patients from the Medicine department; the majority (73%) were male. Eighty percent of patients were hospitalized. Overall mortality was four (3.3%). The various samples collected were blood in duplicate (124), pleural fluid (69) and sputum (55).

Comparison of automation and conventional methods

One-hundred-twenty-four compliant pairs of BacT/Alert 3D and BHIB blood culture sets were evaluated. Forty single isolates were detected in matched pairs. Thirty-four isolates (27.4%) were classified as clinically significant. Six were classified as probable contaminants, micrococci (4) and diphtheroids (2).

Among the 34 clinically-significant single isolates recovered from matched pairs, 22 (65%) were recovered from the automated system and six (18%) each were from both systems and from BHIB alone. The automated system resulted in 22/124 (18%) true positives, along with 1/124 (0.8%) false positives and false negatives each. Conventional method resulted in 6/124 (4.8%) true positives, 11/124 (8.8%) false positives and 5/124 (4%) false negatives. Contamination was found in three (2.4%) of the BacT / Alert 3D exams and in 11 (8.8 %) of the conventional cultures. *Streptococcus* spp., *Klebsiella pneumoniae*, *Streptococcus pneumoniae* and *Haemophilus influenzae* were isolated more frequently in the automated system (Table1). The average time for detection of pathogens was 16.6 hours (range 11.6 – 40 hrs) by the BacT / Alert 3D system compared to 48 hours (range 36 – 72 hrs) by the BHIB method.

Spectrum of CAP

The most-frequently encountered pathogens were *S. pneumoniae* (35.3%) *S. aureus* (23.5%), *K. pneumoniae* (20.5%), *H. influenzae* (8.8%), and *Streptococcus* group A & G (5.8% each).

Antimicrobial sensitivity

The antimicrobial sensitivity pattern by disc diffusion of isolated pathogens is depicted in Table 2; *S. pneumoniae* was 100% sensitive to penicillin, cefotaxime, cefipime, and vancomycin, 92% to ciprofloxacin and 75% to erythromycin. Oxacillin resistance was observed in 2/12 of these isolates. MIC by E-strip method is depicted in Tables 3 and 4**.** Three of the 12 *S. pneumoniae* isolates showed penicillin resistance (MIC= 0.19, 0.25, 0.38 μg/mL) and erythromycin resistance was found in two strains (MIC = 1.32 μg/mL).

MIC Testing revealed two of three *H. influenzae* strains with MIC to penicillin 16 μg/mL and > 256 μg/mL. Both these isolates produced beta lactamase. Two of eight *S. aureus* strains were oxacillin resistant, with MIC = 64 μg/mL. Out of seven *K. pneumoniae*, only one was resistant to cefotaxime (MIC > 256 μg/mL) and another was an ESBL producer. All the isolates were sensitive to cefepime (MIC ≥ 2 μg/mL).

Discussion

Community-acquired pneumonia remains a leading infectious killer. Therefore, expedient etiological diagnosis is fundamental to guide therapy. Diagnosis based on blood-culture positivity depends upon the reliability of the method employed.

Automation had a four-times higher rate of isolation compared to the conventional blood culture method. The time to detection was also decreased (16.6 *versus* 48 hours). This is comparable to previously published reports on septicemic cases [9,12]. The overall bacterial yield in CAP samples was (27.4%), which is within the range reported by others (10-30%) [6]. A greater number of *S. pneumoniae*, *K. pneumoniae*, and *Streptococcus* Group A and G strains were recovered from the automated system. Differences in medium composition and incubation conditions account for the increase in organism retrieval. Bac T/Alert 3D bottles have an enriched medium containing a lower concentration of sodium polyanethol sulphate (SPS) (0.02%). The high concentration of SPS (0.035%) and anticoagulant in BHIB inhibits pathogenic *Neisserriae* and *Streptococcus* spp. [9,10,12].

The false negative BHIB cultures, on final subculture, yielded *S. pneumoniae* (2), *H. influenzae* (2), and *Streptococcus* Group G (1). This is because fastidious organisms, low blood volumes and low bacterial counts may not reach the growth threshold. Only one (0.8%) false negative culture with the Bac T/Alert 3D system yielded diphtheroids; thus no pathogen was missed. Likewise fewer false positive cultures (0.8%) occurred with Bac T/Alert 3D [11]. The false positive signal is due to high leukocyte counts and the inability of a particular pathogen to grow under the conditions of

Table 1. Comparative recovery of pathogens using the Bac T / Alert 3D (B) and Brain Heart Infusion Broth (BHIB) methods.

	Number of isolates recovered with			
	Total	**B only**	**BHIB only**	**B & BHIB**
Streptococcus pneumoniae	12	8	3	1
Staphylococcus aureus	8	4	1	3
Klebsiella pneumoniae	7	4	2	1
Haemophilus influenzae	3	2	0	1
Streptococcus Group G	2	2	0	0
Streptococcus Group C	2	2	0	0
Total	34	22	6	6

Table 2. Antimicrobial sensitivity pattern of pathogens by disc diffusion

Organism*	**N****	**P**	**E**	**Va**	**Cp**	**Ce**	**Cpe**	**G**	**Ak**	**Ox**
Streptococcus pneumoniae	12	12	9	12	11	12	12	9	-	2
Staphylococcus aureus	8	6	5	8	6	8	8	-	-	6
Klebsiella pneumoniae	7	-	-	-	7	5	7	7	7	-
Haemophilus influenzae	3	1	0	3	3	3	3	-	-	-

* All *Streptococcus* group A and G were sensitive to all the antibiotics. **Antibiotics: P: penicillin; E: erythromycin; Va: vancomycin; Cp: ciprofloxacin; Ce: cefotaxime; Cpe: cefepime; G: gentamicin; Ak: amikacin. N: total no. of isolates.

Table 3. Minimum inhibitory concentration (MIC) by E-strip method for isolates from patients with community-acquired pneumonia in India

MIC* Breakpoint (µg/mL)	**S.** ** *pneumoniae* N=12**		***H. influenzae* N=3**		***S.* Group *A* N=2**		**S. Group N=2**		***St.*** *** *aureus* N=8**		
	P	**E**	**P**	**E**	**P**	**E**	**P**	**E**	**P**	**E**	**Ox**
0.016	7	8	0	0	1	2	2	2	6	5	4
0.025	1	0	0	0	0	-	-	-	0	0	0
0 0.032	0	1	0	0	0	-	-	-	0	0	0
0.038	1	0	0	0	0	-	-	-	0	0	0
0.047	1	1	0	0	0	-	-	-	0	0	0
0.064	0	0	0	0	0	-	-	-	1	0	2
0.128	0	0	0	0	0	-	-	-	0	0	0
0.19	1	0	1	0	1	-	-	-	1	0	0
0.25	1	0	0	0	-	-	-	-	0	0	0
0.38	1	1	0	1	-	-	-	-	-	0	0
1	-	1	0	1	-	-	-	-	-	0	0
2	-	0	0	1	-	-	-	-	-	1	0
4	-	0	0	-	-	-	-	-	-	0	0
8	-	0	0	-	-	-	-	-	-	1	0
16	-	0	1	-	-	-	-	-	-	1	0
32	-	1	0	-	-	-	-	-	-	0	0
64	-	-	0	-	-	-	-	-	-	-	2
128	-	-	0	-	-	-	-	-	-	-	-
256	-	-	1	-	-	-	-	-	-	-	-
Total	13	13	3	3	2	2	2	2	8	8	8

E: erythromycin, P: penicillin, Ox: oxacillin. *interpretation of minimum inhibitory concentration (MIC) as per NCCLS guidelines [13]. ***Streptococcus.* ****Staphylococcus aureus:* two strains were ORSA & all were sensitive to vancomycin (MIC = 0.63-0.5 µg/mL), Cefepime (MIC = 0.5-2 µg/mL), cefotax (MIC = 0.25-2 µg/mL). Only two isolates were resistant to ciprofloxacin (MIC = 2,4 µg/mL respectively).

Table 4. Minimum inhibitory concentration (MIC) by agar dilution method for *Klebsiella pneumoniae* isolated from seven cases of community-acquired pneumonia in India

MIC (µg/mL)	Antimicrobial*			
	Cp	Ce	Cpe	Ak
0.25	6	5	4	-
0.5	1	-	2	4
1	-	0	0	1
2	-	0	1	1
4	-	0	-	1
8	-	-	-	-
16	-	1	-	-
32	-	-	-	-
64	-	-	-	-
128	-	-	-	-
256	-	1	-	-

*Cp: ciprofloxacin: Ce: cefotaxime; Cpe: cefepime; Ak: amikacin.

subculture [14]. The greater number (8.8%) of false positive cultures by the conventional method is attributed to difficulties in assessing growth indicators during visual examination.

The percentage positivity of pathogens was 35.3% for *S. pneumoniae*, followed by *S. aureus* (23.5%), *K. pneumoniae* (20.5%), and *H. influenzae* (8.8%). Prior reports from India and other countries have documented an incidence of *S. pneumoniae* ranging from 18% to 51%, *H. influenzae* 6% to 58% and *S. aureus* 15%-17%. The incidence of *H. influenzae* was low and that of the rest of the pathogens was high in our study. Wide variations in the incidence of pathogens are due to differences in age group, comorbidities and prevalent prescribing practices in different areas [3,7,15,16].

Pneumococci were 100% sensitive to cefotaxime, cefepime, penicillin and vancomycin by disc diffusion screening. However, E strip MIC testing showed 25% resistance to penicillin and erythromycin, respectively, and no resistance to cefotaxime. Multidrug resistance (MDR – resistance to penicillin and erythromycin) was seen in 2 of 12 pneumococci. The incidence of penicillin resistance in Asia ranges from 0 to 6% in India to as high as 79.7% in Korea. Fingerprinting analysis revealed mutations in penicillin-binding protein genes, thereby confirming spread of pandemic clones into Asian countries [17-20]. In the United States, MDR *S. pneumoniae* prevalence ranges from 15.3% to 17.6%. This is attributed to selective antibiotic pressure, easy transmissibility, asymptomatic colonization and stable virulence. The therapeutic options left are cefuroxime, and 3rd and 4th generation cephalosporins, as vancomycin is toxic [21,22]. Despite beta-lactam resistance in pneumococci, treatment failures in bacterial CAP have not been seen, but continued surveillance is crucial [23].

Two of three *H. influenzae* were beta-lactamase positive and resistant to penicillin. This is a higher frequency than in a previous Indian study, which reported 23% resistance to penicillin in nasopharyngeal carriers [19]. This is due to the fact that these virulent strains were from invasive disease in this study. Out of eight strains of *S. aureus*, two were oxacillin resistant and two were penicillin resistant. Group A and G *Streptococci* were sensitive to all the drugs tested. Out of seven strains of *K. pneumoniae*, only one was resistant to cefotaxime and was an ESBL producer. This is comparable to previously-published reports on community strains [24,25]. In our study, the bacteria isolated from CAP were 100% susceptible to cefepime. There are no published reports on 4th generation cephalosporin MICs from India [18,19]. A single study from America has shown 96.2 % susceptibility of *S. pneumoniae* to cefepime [22].

In CAP, cefotaxime and cefepime are useful drugs for empirical therapy against MDR strains. The Bac T/Alert system is reliable, time saving and is cost effective. The use of automation is vital in CAP, as rapid diagnosis and effective therapy can considerably reduce mortality.

References

1. Baron E.J., Finegold S.M. Normally Sterile body fluids, bone and bone marrow. In: Forbes B.A., Finegold D.E. and Weissfeld A. eds. Bailey and Scott's Diagnostic Microbiology. Mosby, NewYork. **1998.**
2. World Health Organization. WHO Infectious diseases report graph 5.htm, **1999** accessed on 25.7.2002
3. Berman S. Acute epidemiology of acute respiratory infections in children of developing countries. Rev Infect Dis **1991**;13:S454-62.
4. Broor S., Lalitha M.K., Chaudhary R., et al. Acute lower respiratory tract infections in India. Ind J Med Microbiol **1999**;17:4-9.
5. Levison M.E. Pneumonia, including necrotizing pulmonary infections (lung abscess). In: Fauci S.A., Braunwald E., Isselbacher K.J., et al (eds). Harrison's Principles of Internal Medicine. Mc Graw-Hill, New York. **1998**.
6. Diaz A., Calvo M., O'Brien A., et al. Clinical usefulness of blood cultures in hospitalized patients with community-acquired pneumonia. Rev Med Chil **2002**;130:993-1000.
7. Blondeau J.M., Tillotson G.S. Antimicrobial susceptibility of respiratory pathogens- a global perspective. Seminars in respiratory infections online (Saunders) **2001**;15:1-17.
8. Snyder J.W., Benzing K.S., Munier G.K., et al. Clinical comparison of nonvented aerobic Bac T / Alert blood culture bottle and standard aerobic bottle for detection of microorganisms in blood. J Clin Microbiol **2000**;38:3864-6.
9. Krishner K.K., Whyburn D.R., Koepnick FE. Comparison of the Bac T / Alert Pediatric blood culture system, Pedi-Bac T, with conventional culture using the 20 Millilitre Becton-Dickinson supplemented peptone broth tube. J Clin Microbiol **1993**;31:793-7.
10. Pickett D.A., Welch D.F. Evaluation of the automated Bac T / Alert system for pediatric blood culturing. Clin Microbiol Infect Dis **1994**:320-24.
11. Bourbeau P., Riley J., Heiler B.J., et al. Use of Bac T / Alert blood culture system for culture of sterile fluids other than blood. J Clin Microbiol **1998**;36:3273-7.

12. Lakshmi V. Culture of body fluids using the Bac T/ Alert system Indian J Med Microbiol **2001**;19:67-72.
13. National Committee for Clinical Laboratory Standards. Performance Standards for Antimicrobial Susceptibility Testing, 14th informational supplement M100-S14, **2004**, Wayne, Pa.
14. Martinez R.M., Partal Y., Cases J., et al. An infrequent cause of false positive blood cultures. Clin Microbiol Newsletter **1993**;15:1.
15. Nascimento-Carvalho C.M.C. Etiology of childhood community acquired pneumonia and its implication for vaccination. Braz J Infect Dis **2001**;5:82-97.
16. Bansal S., Kashyap S., Pal L.S., Goel A. Clinical and bacteriological profile of community acquired pneumonia in Shimla, Himachal Pradesh. Indian J Chest Dis Allied Sci **2004**;46:17-22.
17. Invasive bacterial infection surveillance (IBIS) Group, International clinical epicemiology network (INCLEN). Prospective multicentre hospital surveillance of *Streptococcus pneumoniae* disease in India. Lancet **1999**;353:1216-22.
18. Lalitha M.K., Thomas K., Manohoran A., et al. Changing trend in susceptibility pattern of *Streptococcus pneumoniae* to penicillin in India. Indian J Med Res **1999**;110:164-8.
19. Jain A., Kumar P., Awasthi S. High nasopharyngeal carriage of drug resistant *Streptococcus pneumoniae* and *Hemophilus influenzae* in North Indian School Children. Trop Med Int Health **2005**;10,234.
20. Song J.H., Lee N.Y., Ichiyama S., et al. Spread of drug resistant *Streptococcus pneumoniae* in Asian countries ANSORP study. Clin Infect Dis **1999**;28:1206-11.
21. Mera R.M., Miller L.A., Daniels J.J.P., Weil J.G. Increasing prevalence of multidrug-reistant *Streptococcus pneumoniae* in the United State over a 10-year period: Alexander project. Diagn Microbiol Infect Dis **2005**;51:195-200.
22. Pottumarthy S., Fritsche T.R., Jones RN. Comparative activity of oral and parenteral cephalosporins tested against multidrug-resistant *Streptococcus penumoniae* report from the SENTRY antimicrobial surveillance program (1997-2003). Diagn Microbiol Infect Disease **2005**;51:147-50.
23. Metlay J.P. Community-acquired pneumonia: impact of antibiotic resistance of clinical outcomes. Curr Opin Infect Dis **2003**;15:163-7.
24. Ang J.Y., Ezike E., Asmar B.I. Antibacterial resistance. Indian J Pediatr **2004**;71:229-39.
25. Harbarth S., Francois P., Schrenzel J., et al. Community-associated methicillin-resistant *Staphylococcus aureus*, Switzerland. Emerg Infect Dis **2005**;11:962-5.