

## Original Articles

# Comparison of Antimicrobial Susceptibility Testing of Isolates from Blood Cultures by Direct Inoculation Method and PHOENIX

Fang-Lan Yu, Ming-Hwei Lin, Jan-Ching Lee, Yi-Lin Lian  
Chia-Wun Lin, Chi-Tin Chen and Giueng-Chueng Wang

*Department of laboratory Medicine  
Taipei Medical University-Wan Fang Medical Center*

**Background:** Bloodstream infection (BSI) is an important cause of serious morbidity and mortality for hospitalized patients. Empirically Gram stain of bacteria gives the first clue for the etiology of infection and medical treatment. But the delayed treatment on 1 or 2 days after phenotypic identification and drug susceptibility testing may cause potential danger to patients. Rapid drug susceptibility testing can provide earlier information to guide treatment and in less time than bacterial culture and sensitivity testing, for antibiotics therapy.

**Methods:** In this study, we excluded samples of polymicrobial bacteremia. We collected isolates from 815 infection episodes caused by *Escherichia coli* (57%), *Klebsiella pneumoniae* (20.16%), *Enterobacter cloacae* (6%), *Ps.aeruginosa* (9.1%), *Stenotrophomonas maltophilia* (3.1%), and *Acinetobacter baumannii* (3.1%) in a 10-month period. We identified those bacteria with direct susceptibility test with the use of Phoenix100 (BD) during a 10-month period.

**Results:** The results of direct susceptibility were concordant (99%-100%) with those obtained from Phoenix100.

**Conclusion:** These results have the potential to guide clinicians to initiate an early antimicrobial therapy in febrile patients with sepsis shock.

**Key words:** bloodstream infection, bacteremia, direct susceptibility test

## Introduction

Bloodstream infections are life-threatening conditions which require the timely initiation of antimicrobial therapy [2]. For the patient safety, empirical antimicrobial therapy is usually initiated in ill patients and continued for several days before final available results of drug susceptibility from bacterial culture and sensitivity (C & S) tests. Shortened time in microbiological diagnosis of bloodstream infection is important [1,2], to enable patients to receive pathogen-based antimicrobial therapy adequately at an early stage for improving treatment outcome [3,4].

Traditional method of bacterial C & S tests includes pathogen growth, purification and isolation as well as identification and drug susceptibility test. The whole method needs one or two days to finish the report. But clinicians need quick and reliable results for initialing antibiotics therapy or taking necessarily preventative steps. Thus, developing a faster way to classify the microorganism(s) that presented within positive blood culture bottles would guide empirical antibiotic therapy more correctly, and reduce patients' exposure to ineffective or unnecessary antibiotic(s) while awaiting susceptibility test results from bacterial C & S tests [5-7].

In this study, we aimed to focus on the direct sensitivity of blood cultures of Gram-negative bacteria (GNB). Based on the data of the empirical therapy in clinical experience and the discussion with infective

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Corresponding author : Giueng-Chueng Wang, Department of laboratory Medicine  
Taipei Medical University-Wan Fang Medical Center

control committee of the hospital, we used 12 commonly used antibiotics for direct susceptibility, then input the initial report into the laboratory information system (LIS). Clinicians treat the patients with antibiotics after phlebotomy, shortly after their being notified of Gram stain results [4-6]. The results of Gram-positive cocci (GPC) were not discussed in this paper here because GPC often yield unsatisfactory results [16-25] and GNB in blood culture are rarely contaminated. The most common bacteria of positive blood culture in GPC is coagulase-negative staphylococci, which are mostly the contaminants growing simultaneous with other organisms. In this study, we also excluded the strains of GPC (like *Streptococcus pneumoniae*) due to their slow growing and discordant incubating time for detecting and analyzing the different species zone size of AST.

## Methods

The proposal of the study was to investigate the effect of direct drug sensitivity: Blood samples were directly inoculated onto Mueller-Hinton agar plate for detecting susceptible zone, then recorded the results and compared the final data of minimum inhibitory concentration (MIC) results from BD phoenix100. Analyzing the results as proposal, it may reduce the hospitalization time, provide more solution on patients, therefore improve the effect and outcome of therapy [16-19].

## Source and sample management

In this study, we included 815 isolates GNB samples from positive blood cultures (BACTEC Aerobic/F, Anaerobic/F, and Peds/F; Becton Dickinson), but excluded isolates of GPC, yeasts, anaerobic bacteria, contaminants, polymicrobial bacteremia, and others fastidious pathogens.

We collected those isolates from August 1, 2008 to Jun 30, 2009. First we stained inoculums from the blood culture fluid. Then the Gram stain results were delivered to the staff at nursing station or clinician by telephone. Samples were executed direct susceptibility test, direct identification, then inoculated little sample for bacterial C & S test onto the selective agar (BBL BP/EMB; blood plate/eosine Y methylene blue agar plate) for enrichment, using streak technique for separating and sub-culturing to single colony. Incubator condition was 5%CO<sub>2</sub>, 37°C, and overnight growing.

## Provisional direct identification and direct susceptibility test(DST)

Direct identification was performed by direct inoculation, using triple sugar iron agar (TSI) 5 tubes: included triple sugar iron agar slant, lysine iron agar slant (LIA), motility indole ornithine medium (MIO), citrate medium, and urea medium (Bactec, BD). Meanwhile we did the direct sensitivity tests with 2 ml of blood to spray onto the M-H agar plate (14 mm in diameter). The first-line or primary AST paper discs were attached onto M-H plate, including amikacin (AN-30), ampicillin (AM-10), cefazolin (CZ-30), cefmetazole (CMZ-30), amoxicillin-clavulanic acid(AMC-30), cefoperazone (CFP-75), cefotaxime (CTX-30), piperacillin-tazobactam (TZP-110), trimethoprim-sulfamethoxazole (SXT), floximef (FLO), gentamicin (GM), isepamicin (ISP) in which tests used for GNB strains.

## Automatic system BD Phoenix 100 (P100)

The following routine microbiological tests including bacterial identification and drug susceptibility tests (MIC method) were performed using Phoenix100 [BD phoenix P100 auto-analyzer] according to manufacturer's instructions.

## PHX Identification system and software version

The negative-identifying (NID) panels were processed in a Phoenix 100 ID/AST system.

## Data analysis

The comparative results or data were recorded in BD Epicenter system for cumulative analysis, the agreement and discrepancies and predictive value were discussed as followed. For each drug the following measurement of accuracy were used: Category (susceptible, intermediate, resistant [SIR]) agreement matching between the two systems. We classified errors as major error (ME), or false resistant Phoenix result; and minor error (mE), i.e., one system reporting an intermediate result and the other reporting a susceptible or resistant result. In calculating the error rates, we used the following denominators, the number of reference susceptible isolates for ME rate,



and major discrepancies (ME) occurred at ampicillin ( $n=3$ ), amoxicillin-clavulanate ( $n=1$ ). Most discrepancies occurred on ampicillin, amoxicillin-clavulanate, gentamicin. The overall minor error rate was 0.045% (4 of 9759). And the major error rate was 0.045%(4 of 9759).

Table 2 shows the agreement between direct and standard inoculation was 99.91%, positive predictive value (PPV) & negative predictive value (NPV) were 99.9% and 99.9%, respectively.

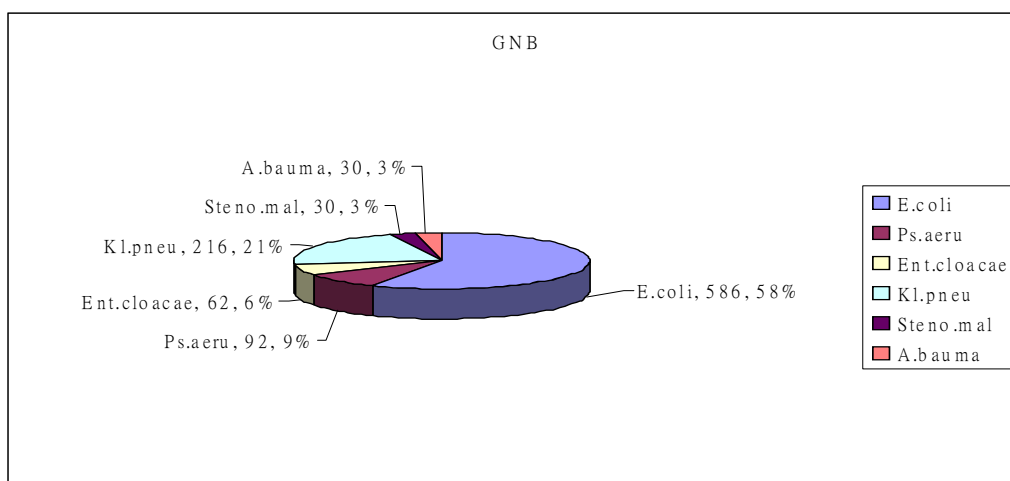
### Discussion

Shortening the time of microbiological analyses for identification and susceptibility testing of bacteria leads to a significant reduced patients' morbidity and mortality as well as the cost expenditure in the hospital[1,2].

Using the Phoenix 100 automatic identify system, as the current standard in comparison with the AST results of direct method is the purpose of the study. To our understanding, some papers discuss and conclude about the results of the blood culture [16-25]. In Coyle et al.'s [21], Fay and Oldfather [23] as well as Doern et al.'s [24] studies, agreements are 92.7%, 94.6%, and 96.8%

individually. The discrepancies of those papers are greater than that in this paper. We suggest those reasons are that the difference timing of inoculum influenced the results, that sampling excluded some polymicrobial bacteremia, and one patient with two or three sets of blood culture resulted in the same organism might increase the agreement, and that positive blood bottles were managed twice or triple a day, at 8:30, 12:00 noon and 16:30 in our study. Like the opinions mentioned above [21], GNB AST results changed less significantly after 4 or 6 hours. We analyzed the 815 GNB isolates, and met the good concordance (99.91%) in AST results. Recently papers discussed about the subject, adding some apparatus and new devices for positive blood samples [16-20] that are labile, and not suitable to us until we find an effective method.

DST errors in two species of *E. coli* and one *Kl. pneumoniae* were ampicillin intermediate (zone size: 16-18 mm in diameter) but P100 were resistant (one *E. coli*, one *Kl. pneumoniae*) or susceptible(one *E. coli*). DST errors in one *E. coli* were AMC resistant (zone size: less than 15 mm in diameter) but P100 susceptible. The discrepancy of major errors between direct sensitivity and automatic susceptibility test were direct results



**Fig.2** The six most ranking of microorganisms in this study( $n=815$ ). *Escherichia coli*( $n=586$ , 57%), *Klebsiella pneumoniae* ( $n=216$ , 20.16%), *Ps.aeruginosa*( $n=92$ , 9.1%), *Enterobacter cloacae*( $n=62$ , 6%), *Steno. maltophilia* ( $n=30$ , 3.1%), *Acinetobacter baumannii*( $n=30$ , 3.1%).

**Table 2** Predictive value of agar-diffusion method and Phoenix100

gold standard/test method	Agar diffusion (+)	Agar diffusion (-)
BD (+)	9763	8
BD (-)	8	9763
		predictive value
sensitivity	99.9	99.9
specificity	99.9	99.9

susceptible but automatic resistant. As to measure the inhibitory zone, some pointlike colonys located in the zone region. Assuming them contaminants from air, we ignored the influence and the data recorded as susceptible. In Barry et al.'s study [25], ampicillin and cephalothin, due to the late development of inner colonies is presumably due to the mutational enzymatic resistance [26]. To our knowledge, it might be the explanation adopted to the discrepancies.

In our study, the six most frequently isolated species ranking were *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *S. maltophilia*, *A. baumannii* and *E. cloacae*, which were similar to those reported previously [6,7,16]. Some papers reported that *P. mirabilis*, *S. marscesnes* are most commonly isolated, too [R & F]. In our statistics, the other organisms were consisting in the most ten ranking (data not shown) and might depend on the prevalence of area. Thinking about the cost and benefit, we suggest that the execution and improvement in AST may be benefit for the farewell for the patients.

The sensitivity agreements (99.91%) of AST results in our study gave us more confidence for the clinical use. It provided the initial referential results in the informative system of the laboratory results, the more useful information for clinical therapy to decrease the misuse of antibiotics. The use of our method can support the clinicians to choose or to modify the empirical therapy acquired from Gram stain results previously, and shorten the length of hospitalization especially the intensive care unit to save the cost.

In the future, we plan to focus on the turbidity modification and to try to use direct appliance (like blood cells removal) in BD phoenix system to evaluate the performance of AST and identification on automatic analyzer, validate the accuracy and increase the outcome [16-18]. Although the Bactec 9240 automatic blood culture systems have reduced the time to detect microorganisms in bloodstream infections compared to empirical therapy, it need more quantitative and qualitative methods to improve and standardize the procedures. The direct sensitivity results agreed with the automatic system BD phoenix 100 mostly. In clinical aspects, it offered the clinicians to choose or modify the therapy acquired only from Gram stain results.

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## 血液檢體直接接種方法及 PHOENIX 測試藥物敏感試驗的比較

余芳蘭 林明輝 李兆清 梁憶林 林嘉玟 陳姿婷 王炯中

臺北醫學大學市立萬芳醫院實驗診斷科

血液感染在醫院常有較高的致死率，以液態培養基培養微生物是目前實驗室標準做法，雖然革蘭氏染色提供治療方向的線索，但在得到鑑定及藥敏結果前的期間卻對病人的生命安全有潛在危險。這研究的目的是為提供比染色結果及培養結果出現前更多的治療參考。直接瓊脂擴散方法是：將血液檢體直接塗抹在培養皿上以及測量微生物藥敏抑制圈，有兩隻菌以上的檢體不在此實驗範圍內。十個月期間總共有815株菌分離出，包括大腸桿菌(57%)、克列博氏菌(20.16%)、陰溝腸桿菌(6%)、綠膿桿菌(9.1%)、嗜麥芽寡養單胞菌(3.1%)、鮑氏不動桿菌(3.1%)被分離出來，並以BD的臨床自動鑑定儀(phoenix)的結果為對照。所得實驗結果和自動化結果相近(99%~100%)，這對於臨床治療的思考以及病人安全照顧提供一個方向。

**關鍵詞：**血液感染(*bloodstream infection*)、菌血症(*bacterimia*)、藥敏(*susceptibility*)