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Direct disk diffusion test during bacteremia: evaluation of antibiotic susceptibility results †

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Abstract: Bacteremia are life threatening emergencies. Early initiation of adequate antibiotic therapy reduces mortality. The purpose of this work was to evaluate the results obtained with the direct AST, carried out directly from positive blood cultures on Mueller-Hinton CHROMagar medium. To do this, 124 strains were tested against 21 antibiotics. The resulting diameters were read after 8h and 18h of incubation, interpreted using the CLSI breakpoints and compared to those obtained with the standard method. The results were extremely satisfactory at 18h (94.43% CA). Non-fermenting GNB recorded the best results with 98.74%CA at 18h. These encouraging results suggest a possible future implementation of the direct-from-blood culture AST as a routine technique.

Keywords: Antibiotic, Bacteremia, Blood culture, Direct AST, Disk diffusion technique

1. Introduction

Bacteremia still constitute a major public health problem. [1-3]. These are absolute emergencies with mortality increasing by 7.6% for every hour spent before the introduction of an adequate antibiotic treatment [4]. However, in almost 40% of cases, the probabilistic antibiotic therapy received is inadequate [5]; hence the interest of its rapid re-evaluation in targeted antibiotic therapy based on the results reported by the microbiology laboratory. It would therefore seem necessary to shorten these deadlines by introducing antibiotic susceptibility tests (AST) that can be carried out directly from positive blood culture bottles. The purpose of this work was to evaluate the results obtained with the direct-from-blood AST and compare them with those obtained with the standard technique.

2. Materials and Methods

It is a prospective study, conducted at the Central Hospital of the Army in Algiers from November 2019 to March 2020 (5 months). All blood-culture bottles that have reported bacterial growth, belonging to BacT/ALERT 3D (bioMérieux) or BD BACTEC FX 40 (Becton Dickinson) system with a delay between the growth signal and the removal of the bottles from the automated incubatory system <18h and a monomorphic appearance of Gram stain in a smear performed directly at from the positive bottles were selected.

The sampling obtained was extended using the inoculation technique described by Sukantha Chandrasekaran et al. [6]. A direct AST was carried out for all the bottles obtained (patients + inoculated) by the method described by CLSI [6], on Mueller-Hinton CHROMagar Orientation medium, which combines presumptive identification and study of the susceptibility of bacteria to antibiotics (figure 1). The choice of antibiotics to be tested was based on the Gram stain results. For Gram-negative bacilli (GNB), the following list was applied: Amoxicillin + Clavulanic Acid (AMC) 20/10µg, Ampicillin (AM) 10µg, Cefazoline (CZ) 30µg, Cefoxitin (FOX) 30µg, Cefotaxime (CTX) 30µg, Ceftazidime (CAZ) 3 0µg, Ceftriaxone (CRO) 30µg, Ertapenem (ETP) 10µg, Meropenem (MEM) 10µg,

Imipenem (IPM) 10µg, Gentamicin (GN) 10µg, Amikacin (AN) 30µg, Tobramycin (TOB) 10µg, Ciprofloxacin (CIP) 5µg, Levofloxacin (LVX) 5µg, Sulfamethoxazole + Trimethoprim (SXT) 1.25/23.75µg. For Gram-positive cocci (GPC), the antibiotics used were: Cefoxitin (FOX) 30µg, Imipenem (IPM) 10µg, Amikacin (AN) 30µg, Gentamicin (GN) 10µg, Erythromycin (E) 15µg, Clindamycin (CC) 2µg, Teicoplanin (TEC) 30µg, Vancomycin (VA) 30µg, Ciprofloxacin (CIP) 5µg, Rifampin (RA) 5µg, Sulfamethoxazole + Trimethoprim (SXT) 1.25/23.75µg. The inhibition diameters obtained were read after 8h and 18h of incubation and interpreted using CLSI breakpoints [7]. Also, a standard AST (reference method) had been carried out for all strains obtained on Mueller-Hinton medium, read and interpreted after 18 hours of incubation; following CLSI recommendations [7]. The results obtained with direct AST were compared to those obtained with standard AST, thus allowing the calculation of the concordance rates (% CA) and disagreements represented by minor errors (% mE), major errors (% ME) and very major errors (%VME); their calculation formulas are described in the CLSI study [6].These results were compared with the acceptance criteria of the FDA for validation of antibiotic susceptibility tests, which states that: the %CA must be > 89.9% and the %ME ≤ 3% [8].

Figure 1. Direct AST of *Klebsiella pneumoniae* on MH CHROMagar Orientation medium, 18h reading

3. Results

During this study, 776 blood-culture bottles were received: 569 were sterile and 207 returned positive, of which 63 met the inclusion criteria previously mentioned. In addition to that, 61 other bottles were obtained by the inoculation technique, thus raising the total number of bottles studied to 124. The distribution of the strains obtained by bacterial species is illustrated in Table 1. An extremely satisfactory concordance rate of 94.43% was recorded at 18h, compared to a lower (87.32%) at 8h. As for disagreements, 78 mE were obtained at 18h against 150 at 8h, 2 ME were recorded at 18h against 12 at 8h. No VME are to be reported at 18h or 8h (Table2).

Analysis of the results by bacterial type shows that all bacterial types reported more than satisfactory concordance rates at 18h (%CA > 89.9%). At 8h, however, this was only observed with non-fermenting GNBs. It was also found that the best results were obtained at 8h as at 18h with non-fermenting GNBs (94.70% CA at 8h and 98.74%CA at 18h). The lowest results were obtained with staphylococci (78.19% CA at 8h and 91.70% CA at 18h). The highest %ME was obtained at 8h and 18h with enterobacteria, while staphylococci recorded the highest %mE at 8h and 18h (Table 3).

The detailed analysis of the results by molecule for all bacteria revealed that a satisfactory concordance rate was obtained at 18 h with most antibiotics tested (17/21) except AMC, CC and TEC due to the high minor errors (mE). At 8h, FDA compliant concordance rates were obtained with only 6 molecules (GN, SXT, LVX, AM, FOX, and AN). concordance rates of less than 89.9% were either due to minor errors (mE) only as was the case for TOB, ETP, CZ, CIP, and AMC or due to minor (mE) and major (ME) errors as was the case for CTX, CRO, MEM, IPM and CAZ. It is also important to note that only CTX, CRO and IPM recorded unsatisfactory rates of ME greater than 3% at 8h, no exceedance of this rate was reported at 18h (Table 4).

Table 1. Distribution of strains found in blood-culture bottles by bacterial species
GNB: Gram Negative Bacilli, GPC: Gram Positive Cocci, CNS: Coagulase Negative Staphylococci

Organism			N	%
GNB n=86 69,35%	Enterobacteria n=65 52,42%	*E.coli*	26	20,97%
		K.pneumoniae	25	20,16%
		S.marcescens	6	4,84%
		E.cloacae	4	3,23%
		E.aerogenes	2	1,61%
		P.mirabilis	2	1,61%
	Non-fermenting GNB n=21 16,94%	*A.baumannii*	13	10,48%
		P.aeruginosa	8	6,45%
GPC n=38 30,65%	Staphylococci n=32 25,81%	CNS	21	16,94%
		S.aureus	11	8,87%
	Enterococci n=6 4,84%	*E.faecium*	6	4,84%
Total			124	100,00%

Table 2. Overall analysis of results obtained with direct AST
CA: Categorical Agreement, mE : Minor Error, ME : Major Error, VME : Very Major Error

Reading delay	CA		mE		ME		VME	
	n	%	n	%	n	%	N	%
8h	1116	87,32%	150	11,74%	12	1,66%	0	0,00%
18h	1357	94,43%	78	5,43%	2	0,24%	0	0,00%

Table 3. Analysis of direct AST results by bacterial type
CA: Categorical Agreement, mE : Minor Error, ME : Major Error, VME : Very Major Error

Bacterial type	Reading delay	CA	mE	ME	VME
		%	%	%	%
Enterobacteria	8h	87,81%	11,00%	2,07%	0,00%
	18h	94,32%	5,45%	0,36%	0,00%
Non-fermenting GNB	8h	94,70%	5,30%	0,00%	0,00%
	18h	98,74%	1,26%	0,00%	0,00%
Staphylococci	8h	78,19%	21,28%	0,87%	0,00%
	18h	91,70%	8,30%	0,00%	0,00%

For enterobacteria, an unsatisfactory overall concordance rate of 87.81% was obtained at 8 h against a more than satisfactory rate of 94.32% at 18 h. As for the discordances, they were mainly represented by the mE (11.00% at 8h and 5.48% at 18h) followed by the ME (2.07% at 8h and 0.36% at 18h). No VME were reported. At 18h, most antibiotics (12/16) had a satisfactory %CA except AMC, CAZ, IPM and CIP. At 8h, however, only 7 molecules (AM, FOX, GN, AN, TOB, LVX and SXT) achieved a concordance rate greater than 89.9%. It is important to note that the majority of ME were reported with antibiotics belonging to the family of β-lactams including CTX, CAZ, CRO, MEM, IPM

at 8h and IPM at 18h. Rates of the latter even exceed the acceptability limit for CTX, CRO and IPM at 8h and CIP at 18h (Table 5).

Table 4. Detailed analysis of direct AST results by molecule of all bacteria combined

CA: Categorical agreement, mE: Minor error, ME: Major error, VME: Very major error, FE: Low number, N: Total number of results obtained per antibiotic molecule, n: Number of concordances or disagreements recorded per antibiotic molecule, ATB: antibiotic, AM: Ampicillin, AMC: Amoxicillin + Clavulanic acid, CZ: Cefazoline, FOX: Cefoxitin, CTX: Cefotaxime, CAZ: Ceftazidime, CRO: Ceftriaxone, ETP: Ertapenem, MEM: Meropenem, IPM: Imipenem, GN: Gentamicin, AN: Amikacin, TOB: Tobramycin, CIP: Ciprofloxacin, LVX: Levofloxacin, SXT: Sulfamethoxazole + Trimethoprim, RA: Rifampin, CC: Clindamycin, TEC: Teicoplanin, E: Erythromycin, VA: Vancomycin

ATB	8H									18H								
	N	me		ME		VME		CA		N	me		ME		VME		CA	
		n	%	n	%	n	%	n	%		n	%	n	%	n	%	n	%
AM	46	1	2.17%	0	0.00%	0	0.00%	45	97.83%	48	1	2.08%	0	0.00%	0	0.00%	47	97.92%
AMC	59	8	13.56%	0	0.00%	0	0.00%	51	86.44%	63	7	11.11%	0	0.00%	0	0.00%	56	88.89%
CZ	61	7	11.48%	0	0.00%	0	0.00%	54	88.52%	65	4	6.15%	0	0.00%	0	0.00%	61	93.85%
FOX	83	5	6.02%	0	0.00%	0	0.00%	78	93.98%	95	2	2.11%	0	0.00%	0	0.00%	93	97.89%
CTX	49	5	10.20%	3	15.00%	0	0.00%	41	83.67%	54	2	3.70%	0	0.00%	0	0.00%	52	96.30%
CAZ	80	12	15.00%	1	2.63%	0	0.00%	67	83.75%	85	8	9.41%	0	0.00%	0	0.00%	77	90.59%
CRO	59	7	11.86%	1	3.85%	0	0.00%	51	86.44%	64	3	4.69%	0	0.00%	0	0.00%	61	95.31%
ETP	61	11	18.03%	0	0.00%	0	0.00%	50	81.97%	65	3	4.62%	0	0.00%	0	0.00%	62	95.38%
MEM	60	13	21.67%	1	1.85%	0	0.00%	46	76.67%	64	5	7.81%	0	0.00%	0	0.00%	59	92.19%
IPM	78	14	17.95%	4	7.02%	0	0.00%	60	76.92%	83	6	7.23%	1	1.64%	0	0.00%	76	91.57%
GN	106	2	1.89%	0	0.00%	0	0.00%	104	98.11%	118	1	0.85%	0	0.00%	0	0.00%	117	99.15%
AN	101	8	7.92%	1	1.20%	0	0.00%	92	91.09%	117	5	4.27%	0	0.00%	0	0.00%	112	95.73%
TOB	78	8	10.26%	0	0.00%	0	0.00%	70	89.74%	83	2	2.41%	0	0.00%	0	0.00%	81	97.59%
CIP	101	20	19.80%	0	0.00%	0	0.00%	81	80.20%	116	10	8.62%	1	1.89%	0	0.00%	105	90.52%
LVX	72	2	2.78%	1	1.92%	0	0.00%	69	95.83%	75	1	1.33%	0	0.00%	0	0.00%	74	98.67%
SXT	92	1	1.09%	0	0.00%	0	0.00%	91	98.91%	106	1	0.94%	0	0.00%	0	0.00%	105	99.06%
RA	22	1	FE	0	FE	0	FE	21	FE	33	1	3.03%	0	0.00%	0	0.00%	32	96.97%
CC	21	10	FE	0	FE	0	FE	11	FE	32	5	15.63%	0	0.00%	0	0.00%	27	84.38%
TEC	22	10	FE	0	FE	0	FE	12	FE	30	8	26.67%	0	0.00%	0	0.00%	22	73.33%
E	24	5	FE	0	FE	0	FE	19	FE	35	3	8.57%	0	0.00%	0	0.00%	32	91.43%
VA	3	0	FE	0	FE	0	FE	3	FE	6	0	FE	0	FE	0	FE	6	FE
TOTAL	**1278**	150	11.74%	12	1.66%	0	0.00%	1116	**87.32%**	**1437**	78	5.43%	2	0.24%	0	0.00%	1357	**94.43%**

Table 5. Detailed analysis of direct AST results for enterobacteria per molecule

ATB	8H									18H								
	N	me		ME		VME		CA		N	me		ME		VME		CA	
		n	%	n	%	n	%	n	%		n	%	n	%	n	%	n	%
AM	46	1	2.17%	0	0.00%	0	0.00%	45	97.83%	48	1	2.08%	0	0.00%	0	0.00%	47	97.92%
AMC	59	8	13.56%	0	0.00%	0	0.00%	51	86.44%	63	7	11.11%	0	0.00%	0	0.00%	56	88.89%
CZ	61	7	11.48%	0	0.00%	0	0.00%	54	88.52%	65	4	6.15%	0	0.00%	0	0.00%	61	93.85%
FOX	61	4	6.56%	0	0.00%	0	0.00%	57	93.44%	64	2	3.13%	0	0.00%	0	0.00%	62	96.88%
CTX	49	5	10.20%	3	15.00%	0	0.00%	41	83.67%	54	2	3.70%	0	0.00%	0	0.00%	52	96.30%
CAZ	60	12	20.00%	1	3.33%	0	0.00%	47	78.33%	64	8	12.50%	0	0.00%	0	0.00%	56	87.50%
CRO	59	7	11.86%	1	3.85%	0	0.00%	51	86.44%	64	3	4.69%	0	0.00%	0	0.00%	61	95.31%
ETP	61	11	18.03%	0	0.00%	0	0.00%	50	81.97%	65	3	4.62%	0	0.00%	0	0.00%	62	95.38%
MEM	60	13	21.67%	1	1.85%	0	0.00%	46	76.67%	64	5	7.81%	0	0.00%	0	0.00%	59	92.19%
IPM	58	14	24.14%	4	8.00%	0	0.00%	40	68.97%	62	6	9.68%	1	1.85%	0	0.00%	55	88.71%
GN	63	0	0.00%	0	0.00%	0	0.00%	63	100.00%	65	0	0.00%	0	0.00%	0	0.00%	65	100.00%
AN	61	4	6.56%	0	0.00%	0	0.00%	57	93.44%	65	2	3.08%	0	0.00%	0	0.00%	63	96.92%
TOB	59	4	6.78%	0	0.00%	0	0.00%	55	93.22%	63	2	3.17%	0	0.00%	0	0.00%	61	96.83%
CIP	59	10	16.95%	0	0.00%	0	0.00%	49	83.05%	63	8	12.70%	1	3.23%	0	0.00%	54	85.71%
LVX	52	1	1.92%	1	2.86%	0	0.00%	50	96.15%	54	0	0.00%	0	0.00%	0	0.00%	54	100.00%
SXT	59	1	1.69%	0	0.00%	0	0.00%	58	98.31%	63	1	1.59%	0	0.00%	0	0.00%	62	98.41%
TOTAL	**927**	102	11.00%	11	2.07%	0	0.00%	814	**87.81%**	986	54	5.48%	2	0.36%	0	0.00%	930	**94.32%**

CA: Categorical agreement, mE: Minor error, ME: Major error, VME: Very major error, FE: Low number, N: Total number of results obtained per antibiotic molecule, n: Number of concordances or disagreements recorded per antibiotic molecule, ATB: antibiotic, AM: Ampicillin, AMC: Amoxicillin + Clavulanic acid, CZ: Cefazoline, FOX: Cefoxitin, CTX: Cefotaxime, CAZ: Ceftazidime, CRO: Ceftriaxone, FTE: Ertapenem, MEM: Meropenem, IPM: Imipenem, GN: Gentamicin, AN: Amikacin, TOB: Tobramycin, CIP: Ciprofloxacin, LVX: Levofloxacin, SXT: Sulfamethoxazole + Trimethoprim.

For non-fermenting GNBs, extremely satisfactory concordance rates of 98.70% and 94.70% were obtained at 18h and 8h respectively. No major or very major errors were reported. Minor errors (mE) were 5.30% at 8h and 1.26% at 18h. For Staphylococci, an unsatisfactory low concordance rate (78.19%) was obtained at 8h. This was mainly due to minor errors (mE) which were 21.28%. At 18h, however, the concordance rate was more than satisfactory (91.70%) and minor errors (mE) were much

lower (8.30%). No major errors were reported at 8h or 18h. For enterococci, no discrepancies were reported.

4. Discussion

Our study recorded at 18h a higher overall %CA than the one obtained in the preliminary study of CLSI [6], it was close to that recorded by the study of Deepashree Rajshekar et al. [9] and the study of Avani Desai et al. [10]. The one obtained at 8h was better than the one recorded by the CLSI at 6h [6]. The global rates of disagreements recorded by our study, particularly at 18h, were acceptable and like those obtained by Deepashree Rajshekar et al. [9] and Avani Desai et al. [10]. Another similarity to the study of Sukantha Chandrase-karan et al. was the decrease in major errors (ME) between early reading and 18h reading. This observation is consistent with the dynamics of diffusion of the antibiotic from the disk: the small measured diameters responsible for major errors are due to an incomplete diffusion of the antibiotic on the agar, hence the interest of adopting specific breakpoints for early readings [6].

In terms of the analysis of results by type of bacteria, we found that Deepashree Rajshekar et al. results' were in the opposite of ours. Indeed, it recorded the highest %CA with staphylococci and enterococci and the lowest with *Pseudomonas*.spp [9]. The differences continue with disagreements: the highest %mE was recorded with staphylococci in our study and with enterobacteria in the study by Deepashree Rajshekar et al. [9]. The same is true for the % VME that was maximum with *Pseudomonas*.spp in the Deepashree Rajshekar et al. study [9] and zero for all categories of bacteria in ours. Finally, the results of the two studies were consistent for the ME that were highest for enterobacteria [9].

Our analysis of results by molecule for all bacteria combined was partly in line with that of the CLSI, which recorded at 6h the best %CA with GN and TOB and the worst with IPM and TGC [6], while our study recorded at 8h its best rates with GN and SXT and the worst with IPM and MEM. Also, it should be noted that CLSI recorded the highest %ME at 6h with antibiotics that are different from ours but belong mostly to the family of β-lactams [6].

Enterobacteria showed an excellent overall %CA at 18h, which was like that obtained in the study by Deepashree Rajshekar et al. [9]. Nevertheless, it appears that the lowest %CA were mainly recorded at 8h as at 18h with β-lactams antibiotics. This same observation was reported by the study of Deepashree Rajshekar et al. with Piperacil-lin-tazobactam and Cefoperazone-sulbacam [9] as well as by the CLSI study [6]. This can probably be explained by the inhibition of the translocation of antibiotics of β-lactam family in bacteria by blood elements present in the inoculum [6].

Particular attention was paid to the main antibiotics used as first-line in the treatment of enterobacterial-induced bacteremia. Our study found very satisfactory %CA for CTX, CRO, ETP and MEM at 18h, thus joining the study by Deepashree Rajshekar et al. concerning CRO and MEM [9]. As well as concordance rates slightly below the acceptability limit for CAZ, IMP and CIP at 18h, this differed from the study by Deepashree Rajshekar et al., who had excellent rates with CAZ and CIP [9]. The study conducted by Avani Desai et al. reported an excellent %CA for C3G [10]. Non-fermenting GNBs had excellent %CA at 18h., like those obtained by Deepashree Rajshekar et al. [9]. In addition, no major or very major errors were recorded at either 8h or 18h, which was better than those reported in the study by Deepashree Rajshekar et al. [9]. Staphylococci also had an excellent %CA at 18h, which was close to that obtained by the study of Deepashree Rajshekar et al. [9]. Also, low %CA associated with a high minor errors were observed for staphylococci at both 8h and 18h with clindamycin and teicoplanin, thus joining in part the study by Avani Desai et al., which does not recommend the communication of clindamycin results to clinicians when performing the direct antibiotic [10]. For enterococci, with a particularly low population, no conclusions could be drawn.

Institutional Review Board Statement: Not applicable

Informed Consent Statement: Not applicable.

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Conflicts of Interest: authors declare no conflict of interest

References

1. Rudd, K. E., Johnson, S. C., Agesa, K. M., Shackelford, K. A., Tsoi, D., Kievlan, D. R., et al. (2020). Global, regional, and national sepsis incidence and mortality, 1990–2017 : Analysis for the Global Burden of Disease Study. The Lancet, 395(10219), 200 211. https://doi.org/10.1016/S0140-6736(19)32989-7
2. Diekema, D. J., Beekmann, S. E., Chapin, K. C., Morel, K. A., Munson, E., & Doern, G. V. (2003). Epidemiology and outcome of nosocomial and community-onset bloodstream infection. Journal of Clinical Microbiology, 41(8), 3655 3660. https://doi.org/10.1128/jcm.41.8.3655-3660.2003
3. Stewardson, A. J., Allignol, A., Beyersmann, J., Graves, N., Schumacher, M., Meyer, R., et al. (2016). The health and economic burden of bloodstream infections caused by antimicrobial-susceptible and non-susceptible Enterobacteriaceae and Staphylococcus aureus in European hospitals, 2010 and 2011 : A multicentre retrospective cohort study. Euro Surveillance: Bulletin Europeen Sur Les Maladies Transmissibles = European Communicable Disease Bulletin, 21(33). https://doi.org/10.2807/1560-7917.ES.2016.21.33.30319
4. Kumar, A., Roberts, D., Wood, K. E., Light, B., Parrillo, J. E., Sharma, S., et al. (2006). Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Critical Care Medicine, 34(6), 1589 1596. https://doi.org/10.1097/01.CCM.0000217961.75225.E9
5. Søgaard, M., Nørgaard, M., & Schønheyder, H. C. (2007). First notification of positive blood cultures and the high accuracy of the gram stain report. Journal of Clinical Microbiology, 45(4), 1113 1117. https://doi.org/10.1128/JCM.02523-06
6. Chandrasekaran, S., Abbott, A., Campeau, S., Zimmer, B. L., Weinstein, M., Thrupp, L., et al. (2018). Direct-from-Blood-Culture Disk Diffusion To Determine Antimicrobial Susceptibility of Gram-Negative Bacteria : Preliminary Report from the Clinical and Laboratory Standards Institute Methods Development and Standardization Working Group. Journal of Clinical Microbiology, 56(3), e01678-17, /jcm/56/3/e01678-17.atom. https://doi.org/10.1128/JCM.01678-17
7. WEINSTEIN, M. P. (2018). M100-performance standards for antimicrobial susceptibility testing, 28th edition. CLINICAL AND LABORATORY.
8. Health, C. for D. and R. (s. d.). Guidance Documents (Medical Devices and Radiation-Emitting Products) - Class II Special Controls Guidance Document : Antimicrobial Susceptibility Test (AST) Systems [WebContent]. Center for Devices and Radiological Health. Consulté 26 octobre 2020, à l'adresse https://wayback.archive-it.org/7993/20180907155335/https://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm080564.htm
9. Rajshekar, D., Chaudhari, K. V., Bhat, P., Prakash, S. S., Raghvan, R., Vasanth, S., et al. (2019). Evaluation of performance of direct disk diffusion test from positively flagged blood culture broth : A large scale study from South India. Journal of Laboratory Physicians, 11(2), 154. https://doi.org/10.4103/JLP.JLP_137_18

10. Desai, A., Unson, E., & Weinstein, M. (2016). Can Direct Disk Diffusion Susceptibility Testing From Positive Blood Cultures Provide Earlier Results to Clinicians? Open Forum Infectious Diseases, 3(suppl_1), 180. https://doi.org/10.1093/ofid/ofw172.47