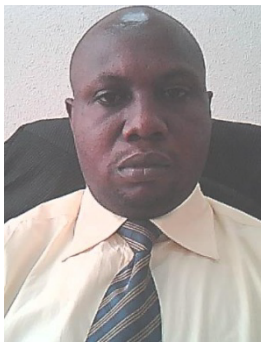
Comparison of the Etest and the routine multi-disc agar diffusion susceptibility of *Staphylococcus* species

***Yah SC[1], Ching FP[2] and Atuanya EI[3]**

Abstract.



Aims: The present study, tend to evaluate the validity and accuracy of Etest as a method for performing *in-vitro* antimicrobial susceptibility testing of *Staphylococcus* with comparison to the routine multi disc agar diffusion. This is because the Etest susceptibility method is not yet known as a rapid, simple reliable technique in developing countries as it combine the functions of both dilution and diffusion technique.

Materials and methods: Ninety-seven *Staphylococcus aureus* and eighty-three *Staphylococcus epidermidis* isolates were obtained from wound samples and identified according to standard morphological and biochemical methods.

The antibiotics susceptibility patterns were determined both by agar disc diffusion and Etest methods in accordance to NCCLS (1997) criteria and manufacturer (AB Biodisk Sweden) respectively.

Results: On the Etest strips, *Staph aureus* was 83.5% sensitive to ciprofloxacin, 52.6% to gentamicin, 48.5% to ampicillin and 8.2% to chloramphenicol while on the multi-disc agar diffusion plates 80.4% of *Staph aureus* were sensitive to ciprofloxacin, 49.5% to gentamicin, 39.2% to ampicillin and 12.4% to chloramphenicol.. On the Etest strips, 80.7% of *Staph epidermidis* were sensitive to ciprofloxacin, 34.9% to gentamicin, 25.3% to ampicillin and 15.7% to chloramphenicol while on the multi- disc agar diffusion plates 89.2% of *Staph epidermidis* were sensitive to ciprofloxacin, 34.9% to gentamicin, 25.3% to ampicillin and 32.5% to chloramphenicol.

Conclusion: The sensitivity patterns between the two methods were essentially similar, however, the Etest method clearly demonstrated intermediate sensitivities which to an extent were absent in routine multi-disc agar diffusion method. Most of the isolates Etest MICs clustered around the sensitive and resistance break points. Etest also demonstrated the MIC and diffusion results on the same strips.

Key words: antibiotic resistance, antimicrobial, gram-positive, chemotherapy.

The emergence of antibiotic resistance against staphylococci, document the need for susceptibility testing to ensure appropriate antimicrobial chemotherapy and therapeutic success.

1. Department of Microbiology, College of Science and Technology, Covenant University, Km 10 Idiroko Road, Ota, Ogun State, Nigeria.
2.Department of Pharmacology, Faculty of Basic Medical Science, Niger Delta University, Yenogua, Bayelsa State, Nigeria
3.Atuanya E I. Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.
*Corresponding author Phone +234803418265: email: yahclar@covenantuniversity.com,

In recent years however, there have been major efforts to improve the spectra of activity of antimicrobials against *Staphylococcus* species. *Staphylococcus* species have traditionally been one of the most significant gram-positive pathogens in major bacterial infections[1]. However, despite their improved activities, newer drug still carry the risk of resistance selection, particularly *Staphylococcus* pathogens that have already intermediate resistance to antimicrobials. This is a clinical problem, especially with methicillin resistant *Staphylococcus aureus (*MRSA) isolates which are widely resistant to quinolones[2,3]. Several testing methods, including agar disc

diffusion, broth microdillution, agar dilution and to a lesser extent Etest have been used to determine the in vitro susceptibilities of *Staphylococcus*, enterococci, *Campylobacter*, etc to antimicrobial agents in some developed countries[4,5]. In Nigeria the Etest interpretive criteria is not yet known as a reliable, simple, rapid determinant of minimum inhibition concentration (MIC) for antibiotic susceptibility testing. Literature search revealed absence of the use of Etest method for susceptibility patterns of clinical isolates generally in Nigeria. The present study, evaluate the validity and accuracy of Etest as a method for performing in vitro antimicrobial susceptibility testing of *Staphylococcus* with comparison to the routine multi disc agar diffusion using isolates from wound samples obtained from the University of Benin Teaching Hospital (UBTH), Edo State, Nigeria.

Methods

Bacterial strains and selection of isolates for analysis

Ninety-seven *Staphylococcus aureus* and eighty-three *Staphylococcus epidermidis* isolates were obtained from surgical wound samples of patients at the University of Benin Teaching Hospital (UBTH). All *Staphylococcus aureus* and *Staphylococcus epidermidis* strains were identified primarily by routine laboratory procedures[6] by their Gram reaction, morphology, mannitol fermentation, catalase, coagulase and DNase production.

Antibiotic Sensitivity Testing:

The antibiotics susceptibility patterns were determined both by agar disc diffusion and Etest methods using Oxoid- Mueller Hinton agar (Difco Laboratories, Detroit, Mich) supplemented with 2% NaCl. Filter papers containing ampicillin (30µg), gentamicin (10µg), tetracycline (30µg), chloramphenicol (10µg), ciprofloxacin (5µg), ofloxacin (10µg) and erythromycin (10µg), (Optun Laboratories Nig Ltd., Nigeria) were used. The antimicrobial agents were aseptically placed on the Mueller Hinton agar plates and incubated overnight. The zones of inhibition of the antimicrobials were read in accordance with the NCCLS[7] criteria.

Agar Etest MIC susceptibility testing.

The Etest minimum inhibitory concentration (MIC) susceptibility testing was determined in accordance with the manufacturer's guidelines (AB Biodisc Sweden). Mueller Hinton agar plates supplement with 2% NaCl were inoculated by swabbing evenly in three directions with 0.5 McFarland standards of the test isolates. The Etest strip (stored in the refrigerator at 4^0C) was applied to each plate with sterile forceps with lowest concentration toward the center of the agar plate. The plates were then incubated at 30 to 35 °C for 24 hours. The Etest MIC values were read directly from the Etest strip MIC scale. The concentration gradient of each antimicrobial agent on the Etest strips was 0.016 to 256µg/ml with the exception of ciprofloxacin and ofloxacin for which the gradient ranged from 0.002 to 32µg/ml.

Results

A total of 180 *Staphylococcus* isolates of two species were obtained from contaminated wounds of patients attending University of Benin Teaching Hospital (UBTH), Benin City Nigeria. The isolates consist of 97 strains of *Staph aureus* and 83 strains of *Staph epidermidis* (Table 1). Apart from *Staphylococcus* species other species encountered in the study were *Pseudomonas aeruginosa, Escherichia coli, Klebsiella, Proteus, Acinetobacter, Enterococcus* etc. the wounds were regarded as infected when purulent discharge occurred or the wound failed to heal within the healing period[8-10]. There was no significant ($P>0.05$) difference between the rate of contamination of wounds of *Staph aureus* and *Staph epidermidis.*

The sensitivities and specificities for the various susceptibility tests of the study are shown in Tables 1, 2 and 3. Table 1 shows the percentage agar multi-disc agar diffusion of the *Staphylococcus* species to antibiotics sensitivity patterns. The routine multi-disc agar diffusion showed no significant

difference (P>0.05) between *Staph aureus* and *Staph epidermidis* but had a significant difference (P<0.05) within the quinolones and commonly available old antibiotics such as gentamicin, chloramphenicol, erythromycin, tetracycline and ampicillin of *Staph aureus* and *Staph epidermidis* (Table 1)

Table 1: The Agar diffusion (%) antibiotic susceptibility patterns against Staphylococcus *aureus* and *Staphylococcus epidermidis*

	Percentage susceptibility						
Isolates	CIP(5µg)	OFL(10µg)	TE(30µg)	AM(30µg)	E(10µg)	CHL(10µg)	GN(10µg)
SA	78	93	30	38	41	12	48
n = 97	(80.4%)	(95.9%)	(30.9%)	(39.2%)	(42.3%)	(12.4%)	(49.5%)
SE	74	65	24	21	26	27	29
n = 83	(89.2%)	(78.3%)	(28.9%)	(25.3%)	(31.3%)	(32.5%)	(34.9%)

Key: SA= S.aureus, SE= S. epidermis,CIP = ciprofloxacin, OFL = ofloxacin, TE = tetracycline, AM= ampicillin, E= erythromycin,CHL= chloramphenicol and GN = gentamicin

Tables 2 and 3 show the Etest susceptibility patterns of *Staph aureus* and *Staph epidermidis* respectively with MIC ranges of 0.02µg/ml to ≥32µg/ml. The Etest susceptibility method gave a more specific elaborate spectrum than the agar disc diffusion method. In Table 2, the MIC values varied along the various concentrations with each antibiotic having its own MIC break points. The Etest strip results showed that 83.5% of *Staph aureus* were sensitive to ciprofloxacin, 52.6% to gentamicin, 48.5% to ampicillin and 8.2% to chloramphenicol (Table 2). Also the Etest strip results showed that 80.7% of *Staph epidermidis* were sensitive to ciprofloxacin, 34.9% to gentamicin, 25.3% to ampicillin and 15.7% to chloramphenicol (Table 3). The *Staph aureus* were 10.3% intermediate sensitive to ciprofloxacin, 19.6% to gentamicin, and 20.6% to chloramphenicol as shown in Table 2. The MIC results also showed that 80.7% of *Staph epidermidis* were sensitive to ciprofloxacin, 34.9% to gentamicin, 25.3% to ampicillin and 15.7% to chloramphenicol as shown in Table 3. The intermediate sensitivity result also showed that 12.1% of *Staph epidermidis* were partially sensitive to ciprofloxacin, 27.7% to gentamicin, and 26.5% to chloramphenicol (Table 3). The results showed that there was no significant differences between (P>0.05, paired t test) the Etest susceptibility and routine multi-disc agar diffusion susceptibility testing methods of the *Staph aureus*. *Staph epidermidis* showed a significant difference at P<0.05 (paired t test) between the two methods. The high specificity of Etest method among the sensitivity ranges (S = sensitive, I = intermediate and R = resistant) was highly appreciative than the disc diffusion method. Plate 2, showed total resistance of Staphylococcus aureus to ampicillin while plate 1, showed resistance of Staphylococcus aureus to ampicillin at MIC > 4µg.

Table 2: The Etest minimum inhibition Concentration (MIC) of antimicrobials against *Staphylococcus aureus*

	Percentage susceptibility		
Antibiotics	% Sensitivity	%Intermediate Sensitivity	% Resistance
CIP (0.002-32µg/ml)	81(83.5)	10(10.3)	6(6.2)
OFL (0.002-32µg/ml)	*34(35.1)	*59(60.8)	4(4.1)
TE (0.016-256µg/ml)	*7(7.2)	*49(50.5)	41(42.3)
AM (0.016-256µg/ml)	47(48.5)	-	50(51.5)
E (0.016-256µg/ml)	42(43.3)	29(29.9)	26(26.8)
CHL (0.016-256µg/ml)	8(8.2)	20(20.6)	69(71.1)
GN (0.016-256µg/ml)	51(52.6)	19(19.6)	27(27.8)

Key: CIP = ciprofloxacin, OFL = ofloxacin, TE = tetracycline, AM = ampicillin, E = erythromycin, CHL = chloramphenicol and GN =gentamicin

Table 3: The Etest minimum inhibition Concentration (MIC) of antimicrobials against *Staphylococcus epidermidis*

	Percentage susceptibility		
Antibiotics	% Sensitivity	% Intermediate Sensitivity	% Resistance
CIP (0.002-32µg/ml)	67(80.7)	10(12.1)	6(7.2)
OFL (0.002-32µg/ml)	45(54.2)	22(26.5)	16(19.3)
TE (0.016-256µg/ml)	*4(4.8)	*23(27.7)	57(68.7)
AM (0.016-256µg/ml)	21(25.3)	-	62(74.7)
E (0.016-256µg/ml)	25(30.1)	22(26.5)	36(43.4)
CHL (0.016-256µg/ml)	*13(15.7)	*22(26.5)	48(57.8)
GN (0.016-256µg/ml)	29(34.9)	23(27.7)	31(37.4)

Key: CIP = ciprofloxacin, OFL = ofloxacin, TE = tetracycline, AM = ampicillin, E = erythromycin, CHL = chloramphenicol and GN = gentamicin

Discussion

The diversity of microorganisms and the high incidence of resistant *Staphylococcus* species in wound samples[8,11,12] had given it credence to compare its susceptibility to Etest and routine agar disc diffusion methods. The versatility and feasibility of Etest had made it possible an attractive alternative to conventional diffusion and dilution susceptibility testing[13]. Our results of the normal conventional agar disc diffusion showed that the isolates were sensitive to the quinolones (ciprofloxacin and ofloxacin) while less sensitive to tetracycline, ampicillin, erythromycin and chloramphenicol (Table 1). These results were similar to those earlier reported by Yah *et al*[8]. This is because ampicillin, chloramphenicol, erythromycin and tetracycline are older, commonly used, cheaper and more available than the newer and more expensive, potent generic antibiotics; ciprofloxacin and ofloxacin. Therefore, one would expect that drugs more commonly affected by bacterial resistance in developing countries are generally inexpensive and popular broad-spectrum agents[14-18]. However, the relationship between antibiotic usage and the emergence and spread of resistance is complex. Resistance of pathogens to these available, cheap, older and commonly used drugs would definitely result in high cost of treatment, longer hospital stay and therapeutic failure, which might lead to life-threatening diseases and more deaths[19].

The Etest MIC results were more elaborated than the common conventional routine agar disc diffusion method (Tables 2 and 3). Jane *et al*[20] also found that the two methods appear to be broadly acceptable for routine clinical use in susceptibility testing of *Pseudomonas aeruginosa*. Intermediate sensitive results of routine agar disc diffusion are always reported as sensitive results as compare to the Etest were the MICs results are read directly on the calibrated strips based on the concentrations as sensitive, intermediate and resistant respectively. In routine agar disc diffusion method there may be a lots of errors in interpretation when measuring the diameters of the zones of inhibition by the antimicrobial agent. More so when the zones of inhibition are apparent, the results are always interpreted as sensitive. However, there was no significant difference between ($P>0.05$, paired t test) the Etest and routine multi-disc agar diffusion susceptibility testing methods of *Staph aureus* but there was a significant difference at $P<0.05$ (paired t test) between *Staph epidermidis* using the two methods. Our results also showed that the isolates MICs were clustered near the break point values for both sensitive and resistance MICs values (data not shown). The results also showed that; the combine effect of Etest method for performing susceptibility testing may make a significant difference in the management of wound infections. Based on the current study, Etest susceptibility testing should be encouraged as a desirable rapid method for tracking of resistant isolates from wound sources. Although NCCLS recommend the disc diffusion and MIC determination, the agar dilution method has been proven to be equally good but very laborious than the Etest method. The Etest susceptibility testing is still a novel in vitro method which its experimentation in less developed countries has not been utilized. According to the reports of Manoharan et al[21] the Etest method was in agreements with agar disc, agar dilution and broth dilution methods where they found no significant different between the methods in determining antimicrobial susceptibilities of *Haemophilus influenzae*. This report is still novel in Nigeria because of very limited reports on the validation of Etest references. However, its high cost had limited it use in Nigeria and other developing countries. We strongly recommend the use of Etest sensitivity testing method in Nigeria and other developing countries.

Conclusion

Our results showed that Etest strip method is a reliable, rapid, easy but slightly expensive susceptibility testing technique. It combines the activity of both diffusion and MIC dilution methods with a distinct intermediate sensitivity. The agar disc diffusion method also is a reliable, rapid, easy and inexpensive

but does not combine the two fronts as in Etest and does not have a good distinct intermediate sensitivity. We strongly recommend the use of Etest sensitivity method in research in Nigeria and other developing countries.

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