Antimicrobial susceptibility of Salmonella typhi and Staphylococcus aureus isolates and the effect of some media on susceptibility testing results

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Citation

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Abstract

Aim:
(i) to determine the antibiogram of Salmonella typhi and Staphylococcus aureus isolates. (ii) to quantify the effect of Nutrient and Tryptone Soy agars on susceptibility testing results.

Methodology :
The Kirby Bauer method was used to evaluate the susceptibility of 30 isolates each of Salmonella typhi and Staphylococcus aureus to various antimicrobial agents on Mueller-Hinton agar (recommended medium). Subsequently, the susceptibility testing procedure was repeated on the isolates using Nutrient and Tryptone Soy agars which are commonly used in Ghana but not recommended for the Kirby Bauer method.

Results:
The prevalence of multiple drug resistance as determined on Mueller-Hinton agar was 83.3% for Salmonella typhi and 80% for Staphylococcus aureus. For Salmonella typhi, resistance ranged from 6.7% (gentamicin and amikacin) to 83.3% (cotrimoxazole, ampicillin and chloramphenicol). In the case of Staphylococcus aureus resistance ranged from 16.7% (erythromycin and gentamicin) to 93.3% (penicillin). Overall, the discrepancy in susceptibility results between Nutrient agar and that of Mueller-Hinton agar was 8.9%, while discrepancy between Tryptone Soy agar and Mueller-Hinton agar, was 17.2%.

Conclusion:
The high resistance rates observed for the organisms to some of the drugs underscore the need for susceptibility testing. However, the use of Nutrient and Tryptone Soy agars for the Kirby Bauer method as practiced by some laboratories in Ghana is discouraged.

INTRODUCTION

Antibiotic-resistant organisms lead to increased hospitalisations, health costs, and mortality. Antimicrobial drug resistance has therefore become an important public health concern associated with serious consequences for the treatment of infections ([1,2]). The phenomenon has been attributed to the misuse of antimicrobial drugs which provide selective pressure favouring the emergence of resistant strains. To contain the problem of antimicrobial resistance, the World Health Organization has provided some interventions, one of which includes effective surveillance of antimicrobial resistance among common pathogens. In Ghana, Staphylococcus aureus and Salmonella typhi are common causes of human infections, and are also recognised as pathogens of high public significance ([3]). Accumulated data has shown increasing drug resistance of these organisms in Ghana and many countries, and therefore the need for surveillance ([3,4,5]).

A number of tests are available for susceptibility testing and hence surveillance of antimicrobial resistance. However, the most widely used method is the disc diffusion test, which comprises mainly the Kirby-Bauer and the Stokes methods ([6]). The two methods differ in their standardisation, reading, and control. While the Stokes method permits the use of any media, the Kirby-Bauer method is limited to Mueller-Hinton medium. Additionally, the control and the test organisms for the Stokes method are tested on the same plate whilst for the Kirby-Bauer method, they are carried out on different plates. In Ghana, the Kirby-Bauer method is the main susceptibility method employed in microbiology laboratories ([3]). A survey showed that some laboratories use other media particularly, nutrient and tryptone soy agars in place of Mueller-Hinton, the recommended medium for the Kirby-Bauer method ([3]). At present, there has been no study yet in the country to document the quantitative effect of these non standard media on quality of susceptibility testing results. Since the use of inappropriate media could affect effective surveillance of resistance and ultimately result in ineffective therapy, there is the need for an investigation to help address this problem.

The objectives of the study were to (i) determine the antibiogram of Salmonella typhi and Staphylococcus aureus isolates (ii) evaluate the quantitative effect of nutrient and tryptone soy agars on the quality of susceptibility testing results in the Kirby Bauer method.

MATERIALS AND METHODS

This prospective study was carried out from June to September, 2006 at the Microbiology Laboratory of the Korle Teaching Hospital located in Accra, Ghana. The hospital is the largest of the three tertiary hospitals in Ghana, and a major referral centre not only for Ghana, but other West African Countries. The Microbiology Laboratory receives high numbers of clinical specimens from patients, and the isolation rate of bacteria from specimens is about 2000 per year ([3]). Some of the common organisms isolated from clinical specimens in this laboratory include, Escherichia coli, Staphylococcus aureus, Klebsiella spp., and Salmonella typhi. The main antimicrobial susceptibility testing method employed in this laboratory is the Kirby Bauer method.

A total of thirty isolates each of Staphylococcus aureus and Salmonella typhi collected from various clinical specimens were used in the study. Laboratory tests on isolates involved biochemical identification and susceptibility testing. Firstly, the isolates were identified by biochemical tests, including coagulase test for Staphylococcus aureus, and triple sugar iron, citrate, indole, and urea tests for Salmonella typhi ([7]). Isolates of Salmonella typhi were also confirmed serologically ([7]). Following this, the Kirby Bauer method was employed to determine the antibiogram of the isolates ([8]). Susceptibility testing on each of the isolate was first performed on Mueller-Hinton agar, and then on nutrient and tryptone soy agars. Escherichia coli NCTC 10418 was used as the control organism for sensitivity testing of Salmonella typhi isolates, while Staphylococcus aureus NCTC 6571 was used as the control organism for Staphylococcus aureus.

The laboratory analyses results were analysed in STATA 7.0 to address the objectives of the study. Zone diameters of susceptibility testing results were categorised as sensitive or resistant based on the NCCLS breakpoint criteria ([9]). Following this, comparisms of susceptibility testing results on Mueller-Hinton agar were compared with those of nutrient agar as well as tryptone soy agar. The prevalence rates of drug resistance including multiple resistance (resistance to three or more drugs) of Staphyloccoccus aureus and Salmonella typhi were computed using sensitivity results on Mueller-Hinton agar.

RESULTS

DRUG RESISTANCE OF SALMONELLA TYPHI AND STAPHYLOCOCCUS AUREUS ISOLATES

The prevalence rates of resistance of Salmonella typhi and Staphylococcus aureus isolates to various antimicrobial drugs on Mueller-Hinton agar are reported in Table 1. For Salmonella typhi, no resistance was observed for cefuroxime and cefotaxime, while resistance to the other drugs tested were; gentamicin (6.7%), amikacin (6.7%), tetracycline (76.7%), cotrimoxazole (83.3%), ampicillin (83.3%), and chloramphenicol (83.3%). The trend of Staphylococcus aureus resistance was as follows; erythromycin (16.7%), gentamicin (16.7%), cotrimoxazole (63.3%), tetracycline (76.7%), cefuroxime (86.7%) and penicillin (93.3%). The prevalence of multiple drug resistance (resistance to three or more drugs) was found to be 83.3% for Salmonella typhi and 80% for Staphylococcus aureus.

COMPARISM OF SUSCEPTIBILITY TESTING RESULTS ON DIFFERENT MEDIA

Tables 1 and 2 report on the discrepancies of susceptibility testing results between Mueller-Hinton agar and each of Nutrient and Tryptone Soy agars. At 5% level, significant discrepancies were observed in susceptibility testing results

between Mueller-Hinton agar and each of Nutrient and Tryptone Soy agars. Overall, the discrepancies in susceptibility results of Mueller-Hinton agar and each of Nutrient and Tryptone Soy agars were 9.9% (40/450) and 17.2% (77/450) respectively. Discrepancies in susceptibility results between Mueller-Hinton and Nutrient agars for the various drugs ranged from 3.3 to 26.7% for Staphylococcus aureus, and 10 to 23.3% for Salmonella typhi. Discrepancies in susceptibility results between Mueller-Hinton and Tryptone Soy agars for the various drugs ranged from 6.7-63.3% for Staphylococcus aureus, and 3.3 to 56.7% for Salmonella typhi. In the case of Salmonella typhi, no discrepancies were observed for some of the drugs in comparing susceptibility testing results on Mueller-Hinton agar with Nutrient and Tryptone Soy agars. This occurred with four drugs for Nutrient agar (cefuroxime, ampicillin, cefotaxime, and chloramphenicol) and two drugs for Tryptone Soy agar (cefuroxime and ampicillin).

Figure 1

Table 1: Resistance of and to various drugs

	S. aureus		*S. typhi*	
Antibiotics tested	**n**	**% r**	**n**	**% r**
Erythromycin	5	16.7	n/a	n/a
Tetracyline	23	76.7	23	76.7
Cotrimoxazole	19	63.3	25	83.3
Cefuroxime	26	86.7	0	0
Gentamicin	5	16.7	2	6.7
Penicillin	28	93.3	n/a	n/a
Ampicillin	27	90.0	25	83.3
Cefotaxime	n/a	n/a	0	0
Chloramphenicol	n/a	n/a	25	83.3
Amikacin	n/a	n/a	2	6.7

n= no. of resistant isolates
% r = percentage resistance of isolates

Figure 2

Table 2: Discrepancies of Susceptibility Results Between Nutrient and Mueller-Hinton Agar Media

Drug	*S. aureus*		*S. typhi*	
	N	%D	N	%D
Erythromycin	1	3.3	n/a	n/a
Tetracyline	3	10.0	7	23.3
Cotrimoxazole	5	6.7	3	10.0
Cefuroxime	4	13.3	0	0.0
Gentamicin	8	26.7	3	10.0
Penicillin	1	3.3	n/a	n/a
Ampicillin	2	6.7	0	0.0
Cefotaxime	n/a	n/a	0	0.0
Chloramphenicol	n/a	n/a	0	0.0
Amikacin	n/a	n/a	3	10.0
[a]Overall	24	11.4	16	7.0

[a]Overall discrepancy in susceptibility results between the two media was 9.9% (40/450)
N_1= number of *S. aureus* isolates that showed discrepancies in susceptibility results between the two media.
N_2= number of *S. typhi* isolates that showed discrepancies in susceptibility results between the two media.
% D=Level of discrepancies in susceptibility results.
N/A=not applicable

Figure 3

Table 3: Discrepancy of Susceptibility Results Between Tryptone Soy and Mueller-Hinton Agar Media

Drug	*S. aureus*		*S. typhi*	
	N	% D	N	% D
Erythromycin	3	10.0	n/a	n/a
Tetracyline	4	13.3	8	26.3
Cotrimoxazole	6	20.0	2	6.7
Cefuroxime	2	6.7	0	0.0
Gentamicin	19	63.3	13	56.7
Penicillin	2	6.7	n/a	n/a
Ampicillin	4	13.3	0	0.0
Cefotaxime	n/a	n/a	1	3.3
Chloramphenicol	n/a	n/a	1	3.3
Amikacin	n/a	n/a	8	3.3
[a]Overall	40	19.0	37	15.4

[a]Overall discrepancy in susceptibility results between the two media was 17.2% (77/450)
N_1 = number of *staphylococcus aureus* isolates that showed discrepancies in susceptibility results between the two media.
N_2 = number of *Salmonella Species* isolates that showed discrepancies in susceptibility results between the two media.
% D=Level of discrepancies in susceptibility results.
N/A=not applicable

DISCUSSION

Drug resistance has become a very important public health issue due to the serious threat of multiply resistant pathogens. The escalation in resistance of bacterial pathogens has also made drug susceptibility testing highly

crucial. In this study, the two bacterial pathogens investigated, Staphylococcus aureus and Salmonella typhi showed high levels of multiple drug resistance. This trend has also been reported especially in the developing world ([3,4,5,10]). In contrast, relatively lower levels of multiple resistance rates of Staphylococcus aureus, Salmonella typhi, and other organisms have been reported in developed countries ([11,12]). In developing countries like Ghana, factors such as poor enforcement of drug policies, insufficient control of drug prescribing, easy accessibility to drugs, and lack of infection control promote and favour drug resistance.

In this study, high levels of resistance were observed for penicillin, ampicillin, cotrimoxazole, tetracycline, and chloramphenicol. These are drugs which have been reported as having high percentage resistance for a lot of microorganisms for several years, and the rate of resistance has been rising over the years not only for clinical isolates but also for the normal intestinal flora of the healthy population ([10,13,14]). Conversely, lower rates of resistance were observed for gentamicin, amikacin, erythromycin and cefotaxime. Relatively, these drugs have been on the Ghanaian market for a short period of time as compared to drugs like ampicillin, and therefore may not have been subjected to high use and or misuse. In addition, some of these drugs like amikacin are expensive and may be prescribed for serious infections, thus limiting their usage. Very high resistance rates were observed for Staphylococcus aureus in relation to penicillin (93.3%) and ampicillin (90%). This excessively high resistance is probably due to beta-lactamase production by the organism, which degrades the beta-lactam ring of these drugs.

The outcome of susceptibility testing is known to be influenced by several factors, some of which include the medium used for bacterial culture, type of drug tested, and the type of organism ([7,8]). These factors were also observed to influence susceptibility testing results in this study. The standard medium for the Kirby Bauer method of susceptibility testing is Mueller-Hinton agar. In Ghana, because nutrient and tryptone soy agars are sometimes used as substitutes for Mueller-Hinton, this prompted us to evaluate the quantitative effect of the two media on the quality of susceptibility testing. Previous studies on the subject have compared susceptibility testing on Mueller-Hinton agar with other standard susceptibility testing media such as Oxoid sensitivity test medium and Iso-Sensitest agar ([13,14]). Because nutrient and tryptone soy agars are general purpose media rather than standard susceptibility testing media, there is hardly any data comparing these media with Mueller-Hinton in susceptibility testing. In this study, using nutrient and tryptone soy agars in susceptibility testing introduced a deviation from the correct results in 8.9% and 17.2% cases respectively. The high discrepancy of susceptibility results observed between Mueller-Hinton agar and each of nutrient and tryptone soy agars for majority of the drugs tested raises doubts about the reliability of the latter two media for susceptibility testing. While similarity of susceptibility results have been reported between Mueller-Hinton agar and certain media including Oxoid sensitivity test medium and Iso-Sensitest agar, high discrepancy have been reported for other media such as Wilkins-Chalgren agar ([15,16]). The suitability of culture media for susceptibility testing is often associated with the composition which could affect growth of the test organism or drug activity in various ways ([7,17]). For media of poor suitability such as nutrient and tryptone soy agars, there is usually the presence of antagnostic substances or unsuitable pH that inhibits drug activity ([7]).

While the high resistance patterns observed for the organisms studied underscore the need for susceptibility testing, the findings of the study discourage the use of nutrient and tryptone soy agars in the Kirby Bauer method as practiced by some laboratories in Ghana, due to the considerable error margin these media may introduce into susceptibility results.

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