

Drug Sensitivity Testing Using Counter Diffusion Technique on Methicillin-Resistant *Staphylococcus aureus* in Clinical Isolates within selected Hospitals in Nakuru County

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Abstract: Several infectious bacterial strains have acquired resistance towards most available antibiotics and it's for this reason that, there is need to study drug sensitivity using counter diffusion technique for methicillin-resistant *Staphylococcus aureus* in isolates so that combination therapy can be embraced since the core factor in using the method is the ability to give good synergistic potential among the drugs. The specific objective was to find out the susceptibility pattern for the MRSA organism using the disc diffusion and compare it to the counter diffusion technique. This was a cross sectional study design and simple random sampling was used to select patients to be surveyed from each of the randomly selected six hospitals. A total of 423 samples were used. A standard laboratory technique was used in the study and MRSA screen test and sensitivity testing were also used. The MRSA was 100 % resistant to lincomycin, gentamycin and negative control and 100% sensitive to vancomycin and counter diffusion technique. Duncan Multiple Range Test established that lincomycin, gentamycin and the negative control did not show any significant differences between them, but they varied significantly with the positive control and the counter diffusion treatments. Positive control and counter diffusion also varied significantly. No child was resistant. A comparison of the susceptibility pattern for the MRSA organism using disc diffusion and counter diffusion technique indicated that the mean zone of inhibition using the counter diffusion technique was 27.1mm and was significantly higher than the zones of inhibition by either lincomycin $p < 0.0001$, or gentamycin $p < 0.0001$.

Keywords: Counter diffusion, disc diffusion, antimicrobial agent, resistant, synergy, resistant

1. Introduction

Resistance in methicillin resistant *Staphylococcus aureus* (MRSA) is related to a chromosomal *mecA* gene that specifies the production of an abnormal penicillin-binding protein called PBP2a or PBP21. Penicillin-binding proteins are membrane-bound enzymes, with targets for all β -lactam antibiotics PBP2a has a decreased affinity for binding β -lactam antibiotics resulting in resistance not only to methicillin but also to all β -lactams including penicillins and cephalosporins [9]. The *mecA* gene complex also contains insertion sites for plasmids and transposons that facilitate acquisition of resistance to other antibiotics. Thus, cross-resistance to non- β -lactam antibiotics such as erythromycin, clindamycin, gentamicin, co-trimoxazole and ciprofloxacin is common [15].

Emily [1] points out that *Staphylococcus aureus* have entered the spotlight as a globally pervasive drug-resistant pathogen. While historically associated exclusively with hospital-acquired infections in immune-compromised hosts, the methicillin-resistant form of *S. aureus* has been spreading throughout communities since the 1990s [11].

According to [1] *S. aureus* has developed numerous mechanisms of virulence and strategies to evade the human immune system, including a host of surface proteins, secreted enzymes, and toxins. In hospital intensive care units, the proportion of MRSA-related infections has increased strikingly from just 2 percent in 1974 to 64 percent

in 2004 [11]. Its presence in the community has been rising similarly, posing a significant public health burden. The growing incidence of MRSA unfortunately has been met with dwindling efforts to develop new, more effective antibiotics. The continued emergence of resistant strains of bacteria such as MRSA demands an urgent revival of the search for new antibiotics.

In the USA, MRSA was responsible for an estimated 94,000 life-threatening infections and 18,650 deaths in 2005, which is more than the 16,268 deaths caused by HIV/AIDS in that same period [7]. Recently, MRSA has been identified in animals used for food production and people in contact with these animals [19]. This involves a specific clone, Multi Locus Sequence Type 398, which seems to spread extensively among animals. The finding of this new zoonotic reservoir of MRSA has led to several research initiatives to investigate its implications. [20]

In Nigeria, the widespread use of antibiotics had led to high levels of resistance among bacterial isolates from patients with nosocomial infections [16]. Methicillin resistant *Staphylococcus aureus* strains that emerged by late 1980s have become increasingly present as nosocomial pathogens. At Nairobi Hospital in Kenya, the problem of MRSA was recognized in midyear of 1996 among both in and out patients [10]. Swift action by the hospital infection control committee resulted in no more cases at the end of the year. There is increasing concern regarding the efficacy of many disinfectants on the market. MRSA was found to be

significantly less susceptible than methicillin sensitive *S. aureus* (MSSA) to chlorohexidine digluconate, 'Hibiscrub' and 'Hibisol' [22]. Hand disinfectants containing both alcohol and chlorohexidine ("Hibisol") are more effective against MRSA than scrubs based only on chlorohexidine (Hibiscrub) and should be used in clinical practice. It is therefore important to select appropriate concentration of disinfectant and rationally use them for disinfection and hospital hygiene [3]. There is a possibility that a significant proportion of laboratory or hospital acquired infections may partly be due to the use of ineffective or low concentration of disinfectants. A continuous monitoring of the efficacy of the commonly used disinfectants is necessary in order to minimize the risk of infection by antibiotic resistant microorganisms, which are common in the hospital [4].

The emergence of high levels of penicillin resistance followed by the development and spread of strains resistant to the semi synthetic penicillins (methicillin, oxacillin, and nafcillin), macrolides, tetracycline, and aminoglycosides has made the therapy of staphylococcal disease a global challenge [17]. In many cases, bacterial resistance to antimicrobial agents may considerably complicate treatment. Several infectious strains have acquired resistance toward most available antibiotics, which warrants global surveillance and antimicrobial stewardship in addition to increased research efforts to understand the mechanisms underlying pathogenesis and antimicrobial resistance, [8]. This is especially true in the case of methicillin-resistant *Staphylococcus aureus* (MRSA) which is one of the most prominent pathogens associated with hospital, community, and livestock-associated infections. Therefore, there is need to study drug sensitivity using counter diffusion for methicillin-resistant *Staphylococcus aureus* in clinical isolates so that combination therapy can be embraced since the core factor in using the method is the ability to give good synergistic potential among drugs. Specifically, focusing on a survey of patients admitted in hospitals within Nakuru County.

2. Materials and Methods

Cross-sectional survey research design was used, Mugenda and Mugenda 1999 [12].

Areas of study and population: Nakuru County is currently the fourth largest urban centre in the country, lies about 1,850 m above sea level. It is located in the Great Rift Valley and about 150 Km West of Nairobi, Figure 1. It has a population of 473,288 people [6]. It is a cosmopolitan district with people from different cultures. Nakuru is home to Lake Nakuru, one of the Rift Valley soda lakes, which forms part of the Lake Nakuru National Park. The park has large numbers of flamingoes that can be seen foraging in the shallow lake. Its main economic activities are mainly manufacturing agriculture and tourism [21]

Sampling methods and collection: Procedure for sample collection was done according to [3]. Both nostrils of the subject were sampled with a paired sterile culture swab; Sample was also collected from wounds under aseptic technique.

Isolation and characterization of *S. aureus*

Swabs were kept viable in Amies transport media [17]. Culturing was done by streak plate method on primary culture plates of Blood agar, Chocolate agar and MacConkey agar. Suspected colonies of *S. aureus* from primary culture plates were confirmed, by Gram reaction, positive catalase, Tube coagulase and Deoxyribonucleases (DNase) test. Sub-culturing was done on Mueller Hinton media get the isolate incubated at 37⁰ C for up to 48 hours. Methicillin disc diffusion (5µg), were used and A growth indicated that the strain is methicillin resistant [16] and it is from these findings that further analysis using that MRSA screen assay was conducted.

MRSA Screen Assay Testing

The MRSA Screening test was performed according to the manufacturer's instructions for the kit. The MRSA- screen assay is a 5-minutes slide latex agglutination test based on detection of PBP2a [13]. The method involves extraction of PBP2a from suspensions of colonies and detection by agglutination with latex particles coated with monoclonal antibodies to PBP2a. Colonies that are truly MRSA produced agglutination. Each test done had a negative and a positive control.

Susceptibility testing

Antibiotic susceptibility test was conducted using Kibby-Bauer (Bauer, 1966) method technique and ran parallel to a new method called counter disc diffusion technique, in this technique the edges of the antibiotics of choice are made to touch along their margins and it's in this context that the active ingredients diffuses from each of the drugs to create a zone of high potency that deters growth of the bacteria. It's from this that final interpretation of the finding was made [15]. Data was analyzed using SAS. A simple linear regression analysis was used to study the relationship between the zones inhibited and the technique used. In the model zone was the outcome variable and technique (with three levels: counter diffusion, lincomycin, and gentamycin) was the covariate, with the latter two being compared to the counter diffusion technique

3. Results

The MRSA was 100 % resistant to lincomycin, gentamycin and negative control (filter paper) while was 100% sensitive to counter diffusion and vancomycin (positive control). Duncan Multiple Range Test (DMRT) established that lincomycin, gentamycin and the negative control did not show any significant differences between them, but they varied significantly with the positive control and the counter diffusion treatments (Table 1). Positive control (vancomycin) and counter diffusion also varied significantly.

Table 1: Analysis between Counter Diffusion and KB Techniques

Treatment	Mean ±SD (n=43)
Positive control	28.3023±0.674 ^A
Counter diffusion	27.1395±1.441 ^B
Negative control	8.0465±0.785 ^C
Lincomycin	8.0000±0.787 ^C
Gentamycin	7.8372±0.615 ^C

Means with different superscripts in the same column are significantly different at $P < 0.05$. (Data analyzed by Duncan's Multiple Range Test).

4. Discussion and Conclusion

The counter diffusion technique show immense potential to the medical and scientific world since it will not only assist in helping to treat the infections caused due to MRSA but also form basis of future in-vitro laboratory testing for the combination therapy for other infection since the approach can be used in other antimicrobial agent as well. The synergistic properties of the counter diffusion will open up the world of laboratory diagnosis more so since lots of antimicrobial agents potential have been reduced dramatically or majority are no longer effective against organisms that affect the lives of the most vulnerable in the society a fact further compounded by the fact that those that are suffer most have weakened immune system either due to lifelong medical condition or from acquired immune depressing conditions for example cancer patients, ICU patients, or those patients that have been exposed to lots of antibiotics.

It is a form of testing for the synergy of two or more drugs *in vitro* and the two drugs used gentamycin and lincomycin from aminoglycosides and another from lincosamide group respectively. Gentamycin is an aminoglycoside and it target specifically the initiation /translation complex thus inhibiting protein synthesis while lincomycin targets the enzyme peptidyl transferase involved in peptide bond formation through the addition of amino acid. It stops the process such that it cannot be translocated into the growing chain. The method showed the effect of synergy of two or more kinds of drugs acting on a single sensitive part of bacterial structure the ribosome involved in protein synthesis, since alteration in the target binding protein (TBPs) is responsible for the methicillin resistance in *S. aureus* and they produce PBPs that are low affinity for the β -lactam antibiotics and as a result they are not inhibited except at a relatively high drug concentration which is clinically achievable.

The emergence of high levels of penicillin resistance followed by the development and spread of strains resistant to the semi synthetic penicillins (methicillin, oxacillin, and nafcillin), macrolides, tetracycline, and aminoglycosides has made the therapy of staphylococcal disease a global challenge. [17], suggests that infection caused by bacterial pathogens is a global problem. In many cases, bacterial resistance to antimicrobial agents may considerably complicate treatment. This is especially true in the case of methicillin-resistant *Staphylococcus aureus* (MRSA) which is one of the most prominent pathogens associated with hospital, community, and livestock-associated infections.

A comparison of the susceptibility pattern for the MRSA organism using the normal method and the counter diffusion technique(s) indicated that the mean zone of inhibition using the counter diffusion technique was 27.1mm and was significantly higher than the zones of inhibition by either *lincomycin* (zone=8mm, $t=87.7$, $p\text{-value} < 0.0001$), or *gentamycin* (zone=7.8mm, $t=88.45$, $p\text{-value} < 0.0001$). The *lincomycin* and *gentamycin* gave similar results. Indeed, all

the subjects were highly sensitive using the counter diffusion technique, but were all resistant using the normal method(s). The positive control gave the same results as counter diffusion technique, while the negative control gave results similar to those obtained using *lincomycin* and *gentamycin*, with all the study participants being highly sensitive when using this technique.

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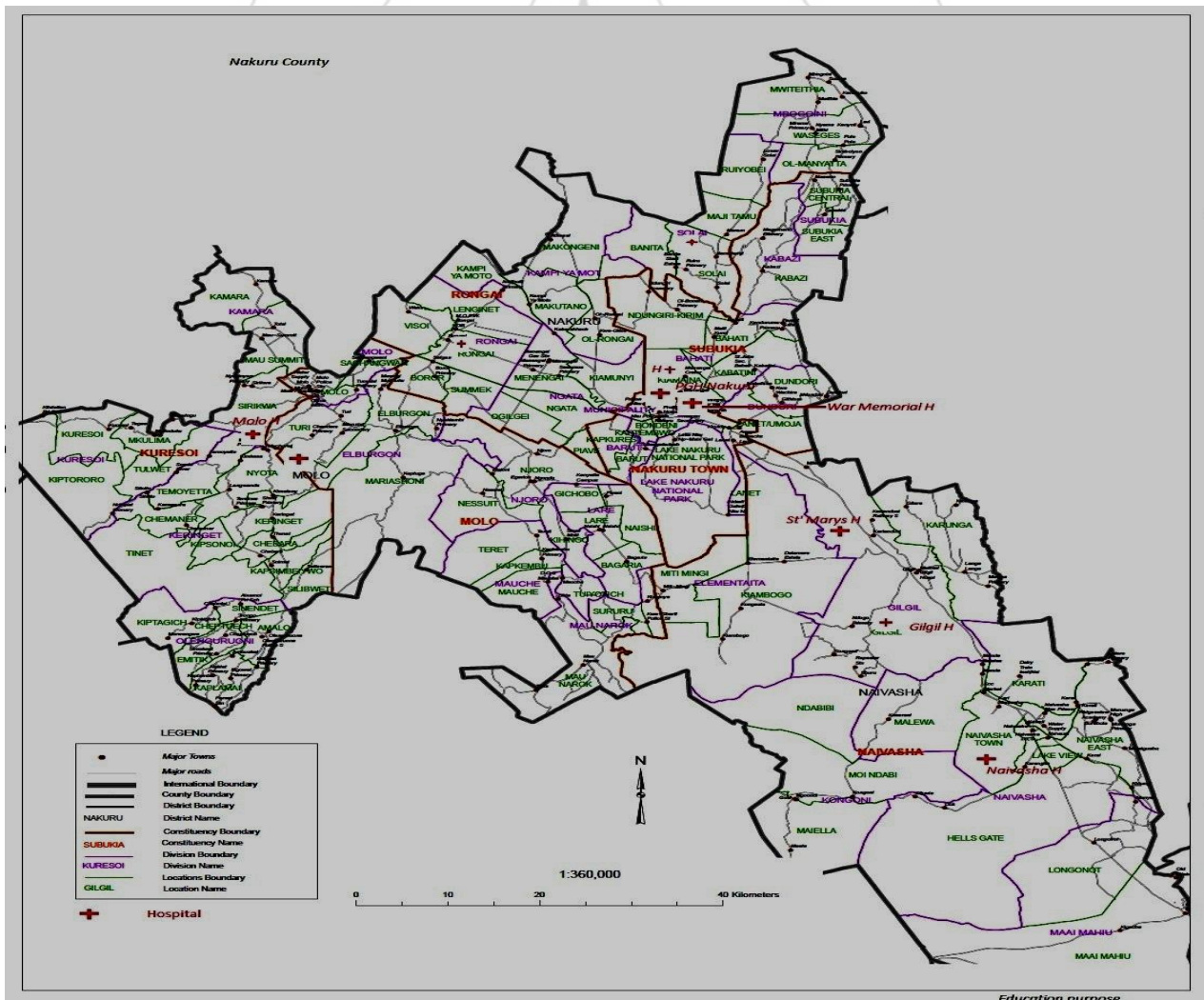


Figure 1: Map of Nakuru county showing area of study, adapted and modified from <http://www.google.com/search?q=nakuru+county+map>