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Methicillin-resistant *Staphylococcus aureus* strains isolated from human and environmental surfaces in a research laboratory

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Abstract

This study aimed to investigate the susceptibility pattern of *Staphylococcus aureus* isolated from human and environmental surfaces in a research laboratory. A total of 320 samples from nostril (n=80), hand (n=80), door knob (n=80) and table surface (n=80) were collected for 16 weeks, before and after work. A total number of 256 samples were found positive for *Staphylococcus aureus*. Out of 80 randomly selected isolates, 50 (62.5%) isolates were resistant to methicillin (MRSA). Hence, the precautionary measures should be taken on self and environmental hygiene as MRSA may be transferred from humans and environmental surfaces.

Introduction

Staphylococcus aureus is usually found on hands, nostrils, tables and doorknobs (Lilenbaum *et al.*, 1998; Onanuga *et al.*, 2005; Rajaduraipandi *et al*., 2006). These microorganisms are one type of facultative anaerobic gram-positive responsible for Staph infections and resistant to the enzyme penicillinase such as Methicillin, Oxacillin and Penicillin. Skin and soft tissue infections are common and range in severity from minor, self-limiting, and superficial infections to life-threatening diseases requiring all resources of modern medicine (Dryden, 2009).

MRSA was first discovered in the 1960s, and quickly became a critical pathogen in hospitals globally, leading to the emergence of healthcare-associated MRSA (HA-MRSA) (Herold *et al*., 1998). MRSA in a research done by Lilenbaum in 1998 was identified from cats giving humans skin diseases. Prevalence of MRSA in the community was reported in Nigeria, even among healthy women (Onanuga *et al*., 2005). David and Daum (2010) further identified and investigated MRSA to be epidemic in communities particularly in specific cities such as Boston, London, Taiwan, Melbourne, Sydney, New South Wales, Queensland, Vancouver, New York, Athens, Cairo, Istanbul, Detroit, San Francisco, Buenos Aires, Atlanta, Kuwait, Shanghai, Kuala Lumpur, Hong Kong, Niigata and Tokyo. Other occurrences of MRSA in hospital settings came from blood tissues, respiratory, bone, joint, endovascular to wound infections (Whitt and Salyers, 2002). In this study, swab samples from human and environmental surfaces in a laboratory were examined for the presence of antibiotic resistant *S. aureus.*

Materials and Methods

Guidelines from the Clinical Laboratory Standards Institute (2002) and Antimicrobial Surveillance Program (1999) were used to guide the investigators on how to collect the samples and carry out antibiogram study.

Swab samples and isolation of S. aureus

The swab samples were taken from a research laboratory using sterile cotton applicator, in the morning and evening (before and after laboratory works). The samples were taken from nostrils, hand, environmental surfaces of the door knob and table surfaces. A total of 320 samples were collected in 16 weeks with 80 samples from each sampling sites.

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Tryptone Soy Broth (TSB) was used as enrichment broth and incubated at 36^0C for 24 hours. The colonies of *S. aureus* were identified through plating method using Mannitol Salt Agar (MSA). Mannitol fermentors such as *S. aureus* appear as yellow colonies with yellow zones in the media.

Antimicrobial susceptibility

A total of 80 randomly selected isolate (20 from each sampling sites) were tested for susceptibility to antibiotics by the disk diffusion method on Mueller-Hinton Agar (MH) according to the guidelines of Clinical and Laboratory Standards Institute (Cockerill *et al*., 2013). Antibiotics tested included tetracycline (30 µg), rifampicin (5 µg), ciprofloxacin (5 µg), oxacillin (1 µg), penicillin (10 µg) and methicillin (5 µg). Initially, peptone water and tryptone soy were used as enrichment broth to promote the growth of *S. aureus*. Mannitol salt agar (MSA) has been used as selective growth media for *S. aureus* to increase the colony count and to get the individual colony to ensure purity. This pure culture of *S. aureus* was then taken with sterile cotton swab and spread evenly into Mueller Hinton agar plate aseptically. Antibiotic discs were placed on the agar using flamed forceps and were gently pressed down to ensure contact and incubated at 37^0C for 24-48 hours. The diameter of "zone of inhibition" around a disk is measured using calipers from the back of the plate with reflected light.

Results and Discussion

The method used to select samples and method used for primary bacterial isolation, are the important factors that should be standardized to allow direct comparison of data between different regions. Meanwhile, the selection of a method is based on many factors such as practicality, flexibility, automation, cost, reproducibility, accuracy, and individual preference. (Dehaumont, 2004). In this study, a total of 256 (80%) out of 320 samples were found to be positive for *S. aureus* including hand, 70 (87.5%); nostril, 80 (100%); door knob, 41 (51.25%); surface tables, 60 (75%). Out of 80 randomly selected isolates (20 from each sampling sites) for antibiogram test, 50 (63.5%) were found resistant to methicillin (hand, n = 20, 100%; nostril, n = 20, 100%, door knob, n = 5, 25%; surface tables, n = 5, 25%). Meanwhile, overall antibiotic susceptibility test result shows that the isolates were resistant towards tetracycline (85%), rifampicin (76%), ciprofloxacin (62%); oxacillin (43%) and penicillin (28%).

The spread of multiple antimicrobial-resistant pathogenic bacteria has been recognized by the World Organization for Animal Health (OIE), the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) as a serious global human and animal health problem. Historically, many infections could be treated successfully according to the clinician's past clinical experience (i.e. empirical therapy); however, this is becoming more the exception than the rule (Walker, 2007). Antimicrobial susceptibility testing (AST) methods involve culturing a sample to obtain a pure isolate and testing to determine which antimicrobial agents inhibit the growth of, or kill the pathogen. The methods may use broth dilution, agar dilution or disk diffusion methods. A number of antimicrobial susceptibility methods and standards are available and their use varies within and between countries (Humphrey, 1952; Cockerill *et al.*, 2013). AST is essential to validate susceptibility to chosen empirical antimicrobial agents, or to detect resistance in individual bacterial isolates. Susceptibility testing of individual isolates is important with species which may possess acquired resistance mechanisms such as members of *Enterobacteriaceae, Pseudomonas, Staphylococcus, Enterococcus* and *Streptococcus pneumonia* (James and Mary, 2009). AST is also an important tool to monitor the emergence and spread of antimicrobial resistance which is transferred between bacteria by horizontal transfer involving the mechanisms of conjugation, transduction and transformation (Aarestrup and Frimodt-Møller, 2011). Moreover, AST provide epidemiological and medical information includes β–lactamases in staphylococci and other gram-negative bacteria.

Staphylococcus, particularly MRSA, has emerged as one of the major global health problem in community and hospitals setting. The study of Mandell *et al.* (1995) proves that MRSA from non-healing wounds came from cross infection or direct contact. However, this increasing incidence can still be curtailed by frequent hand washing (Murray *et al*., 2003). Therefore, it is strongly advised that hand washing in the laboratories and wearing of personal protective equipment or a regular habit of sanitizing door knobs and tables at frequent intervals should be practiced worldwide (Dioso *et al.*, 2014). Chadha (2014) states that there is a need for the development, adoption, and enforcement of appropriate control policies since the infections are resistant to commonly used antibiotics. Regular surveillance including monitoring of antimicrobial susceptibility pattern of MRSA and formulation of a definite antimicrobial policy may be helpful in reducing the incidence of these infections. This is important as MRSA has shown outstanding versatility at emerging and

spreading in different epidemiological settings over time in hospitals, the community, and, recently, in animals (Rossolini *et al*., 2014). Moreover, Schwaber *et al.* (2013) suggested that increasing awareness and implementation of strict infection control protocols are important in order to decrease the risk of infection. In addition, the research on horizontal transmission seemed limited and infection control procedures are insufficient to prevent overt transmission of MRSA on healthcare industries (Uehara *et al*., 2013).

Among food handlers, the ability of *S. aureus* to adhere on the surface of the gloves worn can serve as a source of cross-contamination (Lues and Tonder, 2007). Another contributing factor in *S. aureus* food-borne outbreaks is a cross-contamination in the vicinity of food preparation and processing (Bennett *et al.*, 2008). The finding of high bacterial counts of *S. aureus* in the air and on food contact surfaces in environment is also suggestive of cross-contamination (Kadariya *et al*., 2014). Hence, this study may help the current reporting system as there are very few studies being carried out pertaining to MRSA in environment and human in this region.

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