CHARACTERIZATION OF EXTENDED SPECTRUM β-LACTAMASE (ESBL) FROM Staphylococcus aureus RECOVERED FROM SURGICAL WOUND PATIENTS

BY

OLUTOLA, OLUSAYO TITILOPE
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AUTHOR: OLUTOLA, OLUSAYO TITILOPE

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……………………………….. ……………………………

DR. (MRS.) N. TORIMIRO DR. D.A. AKINPELU
(Project Supervisor) (Head of Department)
DEDICATION

I dedicate this work to the Almighty God, the incomparable and unquestionable God for dealing graciously and mercifully with me.
ACKNOWLEDGEMENTS

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ABSTRACT

This study isolated and identified *Staphylococcus aureus* strains from surgical wounds; determined the antibiotic susceptibility pattern of the *S. aureus* isolates; evaluated the incidence of β-lactamase and extended spectrum β-lactamase (ESBL) production in *S. aureus*. This was with a view to characterizing the ESBL genes in *S. aureus* isolates recovered from surgical wound patients.

One hundred and ten and 107 samples were collected from wounds and anterior nares respectively of subjects at the Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Ile-Ife, Nigeria, using sterile cotton-tipped applicators. The primary isolation media were nutrient agar and mannitol salt agar. The isolates were identified as *S. aureus* based on standard methods. The antibiotic susceptibility typing was conducted using Kirby-Bauer disc diffusion method and interpreted using standard protocol. The acidometric method was used for β-lactamase detection. The induction of β-lactamase was carried out and the enzyme was assayed by the micro-iodometric method. The polymerase chain reaction (PCR) based technique was used for the detection of the resistance genes (*Mec*A, SHV and TEM).

Forty seven (42.7%) *S. aureus* isolates were obtained from 110 wound samples collected while 34 (31.8%) *S. aureus* isolates were also obtained from 107 samples collected from the anterior nares. Forty seven (100%) of the *S. aureus* isolated from wound samples were multiple-resistant while 34 (100%) of the *S. aureus* isolates from anterior nares were also multiple-resistant. β-lactamase production was observed in 14 (41.2%) and 26 (55.3%) of *S. aureus* isolated from the
anterior nares and wounds respectively. The induction of β-lactamase test showed that the enzyme was both constitutive and inductive. MecA gene was detected in 2 (50%) of the methicillin resistant Staphylococcus aureus (MRSA) strains tested and Sulphhydryl variant (SHV) gene was detected in 13 (65%) of the strains tested. BlaTEM gene was not detected in any of the strains. There was no significant difference (p > 0.05) between the resistance patterns of S. aureus isolates from anterior nares and wounds.

The study concluded that the prevalence of multiple-resistant bacterial isolates among surgical wound patients was of epidemiological significance in the control of infectious agents.
CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

The skin is the largest organ in the human body. It is a vital barrier against infection with many defenses to prevent invasion, yet many organisms thrive within the hostile environment (Chiller et al., 2001). Human skin acts as an excellent barrier to infection, provided it is not breached. However, if this barrier is breached, bacteria usually regarded as non-pathogenic on body surface may assume the role of opportunistic pathogens, example some *Staphylococcus* species, *Micrococcus* spp, *Corynebacterium* spp, *Brevibacterium* spp and *Acinetobacter* spp.

A wound is a breach in the skin and exposure of subcutaneous tissue following loss of skin integrity, thus providing a moist, warm and nutritive environment that is conducive for colonization and proliferation of opportunistic and pathogenic microorganisms (Bowler et al., 2001). Colonization of wounds by microorganisms is seen commonly in both the hospital and community settings (Bowler et al., 2001). Wound infections are one of the most common hospital acquired infections and are an important cause of morbidity and account for 70-80% mortality (Wilson et al., 2004; Gottrup et al., 2005).

Development of wound infection depends on the interplay of many factors. Wound infections may occur following accidental trauma and injections, but post-operative wound infections in hospital are most common. Surgical wound infection is clinically defined as purulent discharge from the surgical wound, or spreading cellulitis from the wound (Bowler et al., 2001).

The risk of developing surgical wound infection depends on the number of bacteria that colonize the surgical wound (Dohmen et al., 2009). While the operating wound following surgery is
considered to be “clean”, the surgical wound may be contaminated by air-borne bacteria in the operating room and intensive care units or by bacteria from endogenous sources such as the patient’s mucous membrane, the hands of theatre personnel or by direct contamination by the patient’s normal skin microflora (Kühme et al., 2007). The breaking of the host protective layer, the skin, and thus disturbing the protective functions of the layer, will induce many cell types into the wound to initiate host response (Bowler et al., 2001; Collier, 2003). The severities of complications depend mainly on the infecting pathogen and site of infection (Terry, 1985; Garner et al., 1988).

Staphylococci are Gram positive bacteria with diameter of 0.5µm and could divide in more than one plane to form grape-like clusters (Brock and Frazier, 1996; Harris et al., 2002; Stapleton and Taylor, 2002). The Staphylococci are non-motile, non-spore forming facultative anaerobes that grow by aerobic respiration or by fermentation. Members of this genus are catalase positive and oxidase negative, distinguishing them from the genus Streptococci which are catalase negative and have a cell wall different from that of Staphylococci (Wilkinson, 1997). Staphylococci are tolerant to high concentration of salt and show resistance to heat (Kloos and Lambe, 1991; Wilkinson, 1997). They have a generally benign relationship with their host, however, if the cutaneous organ system is damaged by trauma, inoculation by needles or direct implantation of medical devices (foreign bodies), these organisms can gain entry into the host tissues and may develop life-style of a pathogen (Kloos and Musselwhite, 1975).

The genus Staphylococcus comprises of 41 known species and subspecies that are indigenous to human (Kloos and Bannerman, 1994). Among the 41 species, only five are common in causing human disease such as Staphylococcus aureus, S. epidermidis, S. saprophyticus, S. haemolyticus and S. lugdunensis (Trulzsch et al., 2007). S. aureus is the most virulent specie of the genus Staphylococcus (Murray et al., 2005). Coagulation of blood is used to distinguish S. aureus from
other members of the genus, which are collectively designated as coagulase-negative staphylococci (Ryan and Ray, 2004).

1.1 *Staphylococcus aureus*

*Staphylococcus aureus* forms part of the normal flora of the skin, intestine, upper respiratory tract and vagina (Lowy, 1998). It colonizes asymptomatically the nasal mucosa of about 30% of humans and is responsible for a variety of diseases ranging in severity from mild superficial skin infections to life threatening infections (Lamikanra *et al*., 1985; Lowy, 1998; Waldvogel, 2000; Yamamoto *et al*., 2010). Colonization provides a reservoir from which bacteria can be introduced when host defenses are breached, and it clearly increases the risk for subsequent infections (Kluytman *et al*., 1997; Wertheim *et al*., 2005).

*S. aureus* has been reported to be the most common organism isolated from wound and is the leading cause of bloodstream, lower respiratory tract, skin / soft tissue infections in all regions surveyed (Gorbach, 1996; Diekema *et al*., 2001; Maltezou and Giamarellou, 2006; Godon and Lowy, 2008). It is able to grow and persist in various ways once it adheres to host tissues or prosthetic materials. *S. aureus* can form biofilms (slime) on host and prosthetic surface enabling it to persist by evading host defenses and antimicrobials (Donlan and Costerton, 2002). The invasion of the tissues by *S. aureus* apparently involves the production of a formidable array of extracellular enzymes (invasins) which facilitate the actual invasive process. Some may occur also as cell associated proteins by breaking down primary or secondary defenses of the host which can facilitate the growth and spread of the pathogen (Deresinski, 2005; Godon and Lowy, 2008). The bacterium is a facultative anaerobe and has the ability to regulate its metabolism to withstand drastic changes in environmental conditions (Beaume *et al*., 2010; Todar, 2011). *S. aureus* has evolved many mechanisms to overcome such changes particularly in an infection.