OBAFEMI AWOLOWO UNIVERSITY

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

BY

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(SCP11/12/H/1848)

BEING A THESIS SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF SCIENCE, OBAFEMI AWOLOWO UNIVERSITY, ILE-IFE, OSUN STATE IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (M.Sc.) DEGREE IN MICROBIOLOGY

2015

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

DEGREE: Master of Science (M. Sc.) Microbiology

YEAR: 2015

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CERTIFICATION

This is to certify that this research work was carried out by OLUTOLA, Olusayo Titilope of the Department of Microbiology, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Osun state.

……..	……
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DEDICATION

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

ACKNOWLEDGEMENTS

My unending appreciation goes to God Almighty for everything He hath done in my life and for the grace He has given to me to successfully complete this programme. Lord I say thank you.

I am forever indebted to my supervisor Dr. (Mrs.) Nkem Torimiro who granted me the opportunity to tap from the reservoir of her knowledge. You took time amidst tight schedules to read through this work and provided me with illuminating ideas. May you enjoy promotion and long life in good health in Jesus name, Amen.

I also appreciate the Head of Department, Dr. D. A. Akinpelu who was also my experimental supervisor and a father who is worth commending. Thanks also go to all members of staff of the Department of Microbiology for their support.

I also appreciate the cooperation of staff and patients in adult and children orthopaedic wards, Department of Microbiology and Parasitology, Haematology and General out-patient Department (GOPD), Obafemi Awolowo University Teaching Hospital Complex (OAUTHC) for providing the necessary assistance for this work.

Thanks to Drs. Agboola, Adewale and Okonji of the Department of Biochemistry who provided me with some materials and the privilege to work in the Department, all for the success of this work. May God Almighty bless you all.

I am grateful to my loving parents Mr. and Mrs. Olutola, my elder sister Mrs Bose Oyedele, thanks for being there for me. I am also grateful to Rev. Odedele, Dr. and Mrs Afolabi Adegboyega, uncle Seyi, uncle Seye, for their financial support. Thanks to Adeniran Adeyanju for his assistance, Joshua, Kasang, Seyi, Mary, Niran, Kehinde, Christiana, Seun, Tosin, Amsat, Adekunle, other friends and colleagues.

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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. *Mec*A gene was detected in 2 (50%) of the methicillin resistant *Staphylococcus aureus* (MRSA) strains tested and Sulfhydryl 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 opportunist 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.