PATTERNS AND ANTIMICROBIAL SUSCEPTIBILITY OF BACTERIAL ISOLATES CAUSING PYOGENIC INFECTIONS AT MUHIMBILI NATIONAL HOSPITAL, DAR ES SALAAM, TANZANIA

By

Faustine Engelbert Ndugulile, MD (DAR)

A dissertation submitted in partial fufilment of the requirements for the degree of Master of Medicine in Microbiology and Immunology of the University of Dar es Salaam.

University of Dar es Salaam

March 2001

CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the University of Dar es Salaam a dissertation entitled: *Patterns and antimicrobial susceptibility of bacterial isolates causing pyogenic infections at the Muhimbili National Hospital, Dar es Salaam, Tanzania*, in partial fulfillment of the requirements for the degree of Master of Medicine (Microbiology/Immunology).

.....

Dr Willy K Urassa

University of Dar es Salaam

(Supervisor)

Date.....4th October 2007

.....

Prof Nina Langeland

Center for International Health

University of Bergen

(Second Supervisor)

Date.....26/10/07.....

DECLARATION AND COPYRIGHT

I, Faustine Engelbert Ndugulile, hereby declare that this dissertation is my own original work and that it has not been presented and will not be presented to any other University for a similar or any other degree award.

Signature.....

This dissertation is copyright material protected under Berne Convention, Copyright Act 1966 and other international and national enactments, in that behalf, on intellectual property. It may not be reproduced by any means, in full or in part, except for short extracts in fair dealing, for research or private study, critical scholarly review or discourse with an acknowledgement, without written permission of the Directorate of Postgraduate Studies, on behalf of both the author and the University of Dar es Salaam.

MEDICAL LIBRARY * DAR ES SALAAM *

ACKNOWLEDGEMENT

This thesis was made possible with help, contributions, encouragement and support of a large number of people, perhaps too many to mention each one of them here. However, I would like to thank them for making this possible. First and foremost, I would like to thank my supervisors Dr Willy Urassa and Prof. Nina Langeland for their guidance, patience and constructive criticism throughout the different stages of this work.

I am hugely indebted to the Department of Microbiology at Muhimbili University College of Health Sciences (MUCHS) for having faith in me and providing me an opportunity to train in this field of microbiology.

I wish to thank Prof. Fred Mhalu, Prof. Eligius Lyamuya and Prof. Mecky Matee and Dr Davis Mwakagile for providing me with valuable knowledge in the field of Microbiology and Immunology and also for their encouragement during the course and in preparation of this document.

Many thanks are due to Dar-Bergen collaboration for offering me the scholarship to study Microbiology. My sincere gratitude goes to Prof. Roald Matre for his great assistance while in Bergen, Norway. He made sure that I was comfortable as much as possible miles away from home.

Drs Bjorn Blomberg and Roland Jureen gave invaluable assistance in the laboratory work and also during data analysis. They were with me from the beginning to the end, consulting them at any time and yet they were always ready to assist and advice.

I am grateful to the laboratory staff at the Bacteriology unit at Haukeland Hospital and Muhimbili National Hospital for helping me with the familiarisation of laboratory procedures and tests and helping me when I was stuck.

I am indebted to my parents for their encouragement during the various stages of my academic career. They taught me to aim high and to be none other than the best in whatever I do.

I am also thankful to my brothers and sister for their love and their trust in me.

Lastly, I owe a special debt to my wife, Dr Magdalena Lyimo. She took care of the household while I was away. Her patience and encouragement gave me strength and peace of mind during my work.

DEDICATION

This Dissertation is dedicated to my parents, brothers and sister and lastly to my wife.

ABSTRACT

In order to determine the patterns and antimicrobial susceptibility of bacterial isolate causing pyogenic infections, a cross sectional study was done at the Muhimbili National Hospital, Dar es Salaam from 1st to 31st of September, 1999. Consecutive 209 pus isolates from infected umbilical cord stumps, wounds, post-surgery wounds, ears, eyes and abscesses were collected. The pattern and causative agents of superficial pyogenic infections, characteristics of patients by age and sex, antimicrobial susceptibility patterns of the isolates, comparison of resistance patterns among inpatients and outpatients and relation between antibiotic use and susceptibility patterns were studied.

S. aureus was the commonest isolate (22.5%), of which 87.2% and 2.1% were resistant to penicillin, and methicillin respectively. Among the Gram-negative rods, *Proteus* spp was the most prevalent isolate (12.4%). A significant proportion of Gram-negative bacteria was resistant to ampicillin, doxycycline, amoxycillin/clavulanic acid and chloramphenicol. Pseudomonads were sensitive to all antimicrobials tested. There was no influence of age, sex or patient status in the distribution of the antimicrobial resistant isolates.

The relationship between antimicrobial utilization and resistance was not clearly demonstrated. The resistance to erythromycin among *S.aureus* had increased from 2% in 1979 to 34% in the present study, while tetracycline resistance among *S.aureus* declined from 44% in 1978 to 29.8% in the current study. It was recommended that ampicilin and penicillin should not be used in staphylococcal infections, while tetracycline, trimethoprim/sulphamethaxazole, amoxycilin/ clavulanic acid and erythromycin should be used cautiously basing on sensitivity testing. There is a need to establish a surveillance system to monitor antimicrobial resistance.

MEDICAL LIBRARY

TABLE OF CONTENTS

Certification.....ii

Declaration and Copyright.....iii

Acknowledgement.....iv

Dedication.....vi

Abstract.....vii

Table of contents ix

List of tables.....xii

List of figures.....xiii

Abbreviations.....xiv

1. Introduction and literature review.....1

1.1 Introduction.....1

1.2 Pyogenic infections and consequences.....1

1.3 Causative organisms and clinical spectrum.....2

1.3.1 Wound infections.....3

1.3.2 Post-surgical wound infections.....3

1.3.3 Ear and eye infections.....5

1.3.4 Burn injury.....5

1.3.5 Anaerobic infections.....6

1.4 In-patient and out-patient pyogenic infections.....6

1.5 Diagnosis of pyogenic infections.....7

1.6 Treatment of pyogenic infections.....8

1.7 History of antimicrobial agents and mechanisms of resistance 8

1.8 Antimicrobial susceptibility patterns..... 11

1.9 Factors influencing antimicrobial resistance 13

2. Statement of the problem.....16

2.0 Statement of the problem 16

3. Study objectives..... 17

3.0 Study objectives.....17

3.1 Broad objectives.....17

3.2 Specific objectives.....17

3.3 Hypotheses.....18

4. Methodology.....19

4.1 Study design.....19

4.2 Setting.....19

4.3 Collection of clinical data 19

4.4 Sample collection 20

4.5 Storage of bacterial isolates.....20

4.6 Bacterial identification 21

4.7 Data on antimicrobial consumption 21

4.8 Antibiotic susceptibility testing.....22

4.9 Choice of antibiotic discs for susceptibility testing.....26

4.10 Statistical analysis.....27

4.11 Ethical clearance.....27

4.12 Limitations of the study.....28

5. Results.....29

6. Discussion.....43

7. Conclusion.....55

8. Recommendations.....57

9. References.....58

10. Appendix.....77

LIST OF TABLES

Table 5:1 Age distribution of patients with pyogenic infections.....29

Table 5:2 Prevalence of bacterial species isolated from pyogenic infections.....30

Table 5:3 Common organisms in relation to source of specimen.....31

Table 5:4 The frequency of the bacterial isolates in relation to age.....32

Table 5:5 The frequency of the bacterial isolates in relation to gender.....33

Table 5:6 The frequency of the bacterial isolates in relation to patient status.....35

Table 5:7 Antimicrobial resistance pattern of Gram positive bacteria.....36

Table 5:8 Antimicrobial resistance pattern of Gram negative organisms.....38

Table 5:9 Antimicrobial resistance pattern of Pseudomonads.....38

Table 5:10 Antimicrobial resistance pattern of Staphylococci spp in out-patients and in-patients.....40

Table 5:11 Antimicrobial resistance pattern of Gram negative bacteria in in-patients and out-patients.....41

LIST OF FIGURES

Figure I Antimicrobial consumption calculated as DDD/patient days/1000 42

ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
AMC	Amoxycillin/clavulanic acid
AMP	Ampicillin
AMK	Amikacin
ATCC	American type culture collection
CAZ	Ceftazidime
CHL	Chloramphenicol
CIP	Ciprofloxacin
CLI	Clindamycin
CNS	Coagulase negative staphylococci
CXM	Cefuroxime
DOX	Doxycycline
DDD	Defined Daily Dosage
ERY	Erythromycin
GEN	Gentamicin
GNR	Gram negative rods
GPC	Gram positive cocci
ICU	Intensive care unit
IMP	Imepenem
HIV	Human immunodeficiency virus
MIC	Minimum Inhibitory Concentration

MRSA	Methicillin resistant *Staphylococcus aureus*
NNIS	National Nosocomial Infections Surveillance
NOR	Norfloxacin
OXA	Oxacillin
PEN	Penicillin
PSE	Pseudomonads
SPSS	Statistical Package for the Social Sciences
SXT	Sulphamethoxazole/Trimethoprim
TOB	Tobramycin
USD	United States Dollar
VAN	Vancomycin
VRE	Vancomycin resistant Enterococci

1.1 Introduction and Literature review

Bacterial infections are a major cause of morbidity and mortality in developing countries. Pyogenic infections are common in all ages. These infections are caused by pus producing organisms, mostly involving soft tissues (1). These infections include surgical and burn wounds, pyomyositis, infection of the ears and umbilical cord stumps. Characteristically, the lesions contain pus, which consists of living, dead, or disintegrated neutrophils, macrophages and debris of tissue cells, all suspended in an inflammatory exudate (1).

1.2 Pyogenic infections and their consequences

Pus producing infections can be acquired in hospital setting and also in the community. Hospital acquired infections commonly occur during hospitalization. The sources of such infections are multiple, including contaminated hands of the hospital staff, patients, contaminated equipments and hospital environment. The problem is more common in the developing world compared to the developed world probably due to limited resources and poor hygienic practices in hospitals (2,3).

Hospital-acquired infections increase morbidity and mortality, decrease treatment success, prolong hospitalisation period, reduce hospital turnover rates and increase cost of patient care (4,5). In the

United States, nosocomial infections develop in at least 5% of patients admitted in hospitals (6). They have been reported to result in an extension of hospital stay on average of 4 days, accounting for an estimated 60,000 deaths per year, and adding 10 billion USD per year to the national health care expenditure (7).

Data on the magnitude of nosocomial infections in most developing countries is scarce (8). However, a study done in Trinidad and Tobago between 1992 and 1995 reported a nosocomial infection rate of 10.0 per 100 (10%) admissions. It has been estimated that the costs of extra days stayed due to nosocomial infections was approximately USD 697,000 which was approximately 5% of the annual hospital budget (3).

1.3 Causative organisms and clinical spectrum

Several micro-organisms are capable of causing pyogenic infections, depending on the site of infection. In a study done in Kenya between 1991-95, *Staphylococcus aureus (S. aureus)* was found to be the most predominant species (50.6%) followed by *Escherichia coli (E. coli)* (9.3%), *Proteus* spp (8.4%) and *Klebsiella* spp (8.0%) among patients presenting with pyogenic infections (4). Other microorganisms isolated in that study included *streptococci* (7.6%), *Enterococcus* spp (4.6%) *Citrobacter* spp (3%) and α-haemolytic *streptococci* (2.1%) (4).

Similar results were also reported from another study done in Ethiopia between 1994-95, where it was reported that *S. aureus* was the most predominant species (44.4%) followed by *E. coli* (21.4%), *Proteus* spp (11.2%), *Enterobacter* spp (9.3%), *Klebsiella* spp (6%), *Citrobacter* spp (5%) and *Pseudomonas* spp which accounted for 4.2% of the isolates (5). In a study done by Prabhakar *et al* in 1981 on diabetic gangrene and non-gangrenous foot ulcers, it was observed that there were mixed infections, with predominant organisms being *Enterococci*, *Proteus*, *Pseudomonas* and *Klebsiella* spp (9).

1.3.1 Wound infections

Superficial wound infections whether acute or chronic provide a favourable environment for bacterial colonization and eventually growth. *S. aureus* has been reported to be the commonest organism in superficial infections (10). Others include *Pseudomonas aeruginosa* (*P. aeruginosa)*, coagulase negative staphylococci, group A *streptococci* and *E. coli* (10).

1.3.2 Post surgical wound infections

Post surgical wound infections are still a major problem in the developing and developed countries. The prevalence of these infections is variable. Apart from host factors, post surgical wound infections are influenced by surgical skills and hospital environment

(11). Saha *et al* found that the prevalence of post–surgical wound infection in Bangladesh was 31%; the main causative organisms included *E. coli, S. aureus, Klebsiella pneumoniae (K. pneumoniae), Pseudomonas spp, Proteus vulgaris (P. vulgaris)* and *Streptococcus pyogenes (S. pyogenes)* (12). In North Jordan, Abussaud *et al* documented an overall post-operative wound infection rate of 3.6% that was caused by *S. aureus* (55.5%), *E. coli* (16.6%), *P. vulgaris* (11.1%), while *P. aeruginosa, Enterobacter aerogenes (E. aerogenes)* and *Aeromonas hydrophila* (*A. hydrophila)* each accounted for 5.6% of all infections (13).

In the United States, data on nosocomial infections collected by the National Nosocomial Infections Surveillance (NNIS) between 1986 and 1990 showed that *S. aureus* (17.1%), *enterococci* (13.3%), coagulase negative *staphylocci* (12.6%), *E. coli* (9.4%) and *P. aeruginosa* (8.2%) were the most prevalent organisms in surgical wound infections (14). In Africa, studies done in Nigeria and Ethiopia reported overall post-surgical wound infection prevalence of 4.9% and 21% respectively, with *S. aureus* and Gram-negative organisms being the most prevalent organisms (15,16). In a study done at Muhimbili National Hospital in Tanzania in 1973, it was estimated that 6.6% of all surgical wounds became infected (17).

1.3.3 Ear and Eye infections

In acute ear infections (acute otitis media), *Streptococcus pneumoniae (S. pneumoniae)* and *Haemophilus* spp have been reported to account for about 50% of all infections (19-21). *Moraxella catarrhalis (M. catarrhalis)*, *S. pyogenes*, *Mycoplasma* spp and *S. aureus* have been found in a few patients. In chronic suppurative otitis media, *S. aureus*, coliform bacilli, *Pseudomonas* spp and *Bacteroides* spp are common pathogens (18-21) whereas otitis externa is commonly caused by *P. aeruginosa*, coliform bacilli and *S. aureus* (22). Bacteria that have been reported to cause purulent conjuctivitis include *S. aureus*, *Chlamydia trachomatis (C. trachomatis)*, *Neisseria gonorrheae (N. gonorrheae)* and *Haemophilus* spp (18,23-29).

1.3.4 Burn injuries

Bacterial infections are an important cause of morbidity and mortality among patients with burn injury. Necrotic tissues, protein rich exudates, poor blood supply and diminished immunity provide favourable environment for bacterial colonisation and growth (30). Several studies in developing and developed countries have shown *S. aureus* and *Pseudomonas* spp to be the most prevalent organisms in burn injury infection. Other organisms that are commonly found include *Enterobacter* spp and *Klebsiella* spp (30-36).

MEDICAL LIBRARY

1.3.5 Anaerobic infections

Anaerobic bacteria including *Bacteroides* spp and *Peptostreptococci* have been isolated in pyogenic infections (28,37). However, reported pyogenic infections due to anaerobic organisms are relatively few due to the fact that the isolation of anaerobes is costly, time consuming and often not standardized (38). Besides the requirements of special environment and supplements for isolation, the growth of anaerobes is often slow and the yield is usually low (37,38).

1.4 In-patient and out-patient pyogenic infections

There is conflicting literature regarding the differences in the organisms isolated from in-patients and those from out-patients including their antimicrobial susceptibility patterns. Maselle *et al* in 1980 reported that out-patient organisms were more susceptible to antimicrobial agents than in-patient isolates in Tanzania (39). Malonza *et al* in a community-based study done in Kenya between 1991-95, reported that there was no difference in susceptibility pattern of bacterial pathogens in community and hospital acquired infections (3). Ellison *et al* in a study done in US in 1987, in which he compared susceptibility patterns of out-patients and in-patients, concluded that the patterns were similar (40).

1.5 Diagnosis of pyogenic infections

Diagnosis of pyogenic infections can be done clinically and then confirmed in the laboratory. The colour of the pus coupled with its smell can give a probable identity of the causative agent. For example, blue-greenish pus is suggestive of *Pseudomonas* infection while foul-smelling pus would be suggestive of an anaerobic infection (18). Microscopic examination of pus specimen often gives early and important indication of the nature of the infecting organisms and this may assist in the selection of antimicrobial drugs. Despite the importance of clinical assessment criteria in diagnosing wound infections, the diagnosis still remains non-specific, requiring confirmation by laboratory isolation of the causative organisms. Acute untreated pyogenic infections are characterized by a predominance of polymorphonuclear leukocytes while chronic infections are characterised by the presence of lymphocytes and monocytes. Microscopic evaluation of smears and culture of pus specimens may often give early provisional identity of the possible etiological agent. Aseptic techniques are required to obtain pus specimens for culture and sensitivity testing in order to avoid including body flora that are contaminants (18).

1.6 Treatment of pyogenic infections

Antimicrobial drugs which are used initially in the treatment of infections, are chosen on the basis of clinical impression with a tentative etiological diagnosis on clinical grounds. On the basis of this "best guess", a probable drug of choice can be selected. However, before administering the drugs, specimens should ideally be obtained for laboratory isolation of the causative agents (18). The laboratory results may necessitate selection of a different drug. The identification of certain micro-organisms that are uniformly drug susceptible eliminates the necessity for further testing and permits the selection of optimally effective drugs solely on the basis of experience (18). More commonly, antimicrobial susceptibility testing of isolated micro-organisms is essential. However, in several health care facilities in Tanzania, this is not done due to lack of essential laboratory facilities.

1.7 History of antimicrobial agents and mechanisms of resistance

An antimicrobial agent is any chemical substance that is capable of inhibiting growth or causing the death of micro-organisms. They can either be bactericidal or bacteriostatic. They exert their activity through selective toxicity, by destroying the micro-organisms without causing harm to the body. Antibiotics first came to the forefront with

the discovery of penicillin by Fleming in 1929. He observed that bacteria failed to grow in the vicinity of a colony of a fungus called *Penicillin rotatum* (41). In 1940, Chain and Florey discovered that the above effect was due to a substance called penicillin produced by these organisms (42). During the next 25 years, research on chemotherapeutic agents largely centered around substances of microbial origin called antibiotics. The isolation, concentration, purification and mass production of penicillin were followed by the development of streptomycin, tetracyline, chloramphenicol and many other agents (43). These substances were originally isolated from filtrates of media in which their respective molds had grown. Subsequently, others have been synthesized, and in recent years biosynthetic modification of molecules have been a prominent technique for development of new antimicrobial agents (43).

Mechanism of action of antimicrobial agents is based on selective toxicity. The selective toxicity may be a function of a specific receptor required for drug attachment, or it may depend on the inhibition of biochemical events essential to the organisms but not the host cells (43).

Antimicrobial agents can act by inhibiting cell wall synthesis, which are found on bacterial cell and not human cells. Penicilins,

vancomycin, cephalosprins, bacitracin and cycloserine inhibit various stages in the synthesis of the cell wall, making the bacterial cells fragile and easy to rupture (43). Amphotericin B, colistin, imidazoles, triazoles and polyene act by inhibiting bacterial cell membrane function, utilizing the existing structural differences in bacterial and animal cells (43).

Protein synthesis inhibitors, for example, erythromycin, tetracycline chloramphenicol and aminoglycosides utilize the differences in the ribosomal make up between human and bacterial cells to achieve selective toxicity (43). Nucleic acid synthesis inhibitors, for example, quinolones, trimethoprim, sulphonamides and rifampin act by inhibit various stages which are important in the synthesis of bacterial Deoxyribonucleic acid (DNA) (43).

The antimicrobial resistance may be genetic or non-genetic. Non-genetic resistance includes failure to act on metabolically inert bacteria, intracellular bacteria and those bacteria that have lost the specific target structure for a drug (43). Genetic origin of antimicrobial resistance may be due to spontaneous mutation or due to acquisition of plasmids, which code for enzymes that are capable of destroying drugs. Bacteria have also evolved mechanisms of evading the action of antimicrobial agents, these include production of

enzymes that destroy drugs, for example staphylococci produce β-lactamase which destroy the β-lactam ring in penicillin and hence rendering the drug ineffective (43-45). Bacteria can also alter their outer membranes and hence impairing the permeability of the drug, for example, tetracyclines accumulate in susceptible bacteria but not in resistant bacteria (43). Other mechanisms include altered structural targets for the drug, for example, penicillin resistance in S. *pneumoniae* and enterococci is due to alteration in penicilin binding proteins (43).

Bacteria can also develop an altered metabolic pathway that bypass the reactions inhibited by antibiotics, for example, sulfonamide resistant bacteria can utilize preformed *p*-aminobenzoic acid (PABA) instead of extracellular PABA (43). Bacteria can alter their enzyme producing enzymes which are still functional but which are not affected many drugs, for example, in trimethoprim resistant bacteria, the dihydrofolic acid reductase is inhibited less efficiently than in trimethoprim sensitive bacteria. (43).

1.8 Antimicrobial susceptibility patterns

The increasing prevalence of bacterial resistance to commonly prescribed antimicrobial agents, especially in the developing countries poses a major challenge in the management of bacterial

infections (46-48). The global magnitude of antimicrobial resistance is unknown and more so in the developing countries where the literature is very scanty and in most cases deficient (8). The frequency of resistance to antimicrobial agents for individual pathogens and the patterns of multiple resistances vary between countries and often in different parts of the same country. However, the situation has become worse in the last few years (47-53). Antibiotic resistance pattern is rapidly evolving, hence a need for a constant surveillance.

S. aureus is a major cause of nosocomial infections and it is noted for its high resistance to penicillin and other antibiotics. Currently, more than 80% of the isolates from almost any part of the world have plasmid-mediated resistance to penicillin and ampicillin (54-56). In a study done in Kenya between 1991-95 it was shown that among *S. aureus* isolates, 88.5%, 58.6% and 18.5% were resistant to penicillin, tetracycline and erythromycin respectively (4). Among *S. aureus* strains isolated in Gondar in Ethiopia between June 1994 to May 1995, 72.7%, 46.7% and 17.8% were resistant to tetracycline, penicillin and erythromycin respectively (5). In a study done in 1982 at Muhimbili Medical Centre (MMC), Maselle *et al* showed that among *S. aureus* isolates 90% were resistant to penicillin, while 74.4% and 3.3% were resistant to tetracycline and erythromycin respectively (39). Another study done at MMC by Urassa *et al* in 1998 showed

that among *S. aureus* isolated 93.5%, 62.7% and 31.9% were resistant to penicillin G, tetracycline and erythromycin respectively (57).

1.9 Factors influencing antimicrobial resistance

Development of antimicrobial resistance occurs as a result of complex interactions, which encourage the emergence, persistence and enhance transmission of resistant organisms (49). Development of antimicrobial resistance has been shown to occur in widespread antibiotic use (47,50,52,53). It is estimated that nearly one-half of hospitalized patients in the United States receive antimicrobial agents (58). Several workers have reported a positive correlation between antimicrobial consumption and the increase in resistance of organisms (59-62). In a study done in a Finnish hospital between 1971 and 1984, the trimethoprim resistance pattern of *E. coli* isolates from urine specimen was related to the antimicrobial use (59). Cometta *et al* in a study done on patients with cancer and neutropenia, reported that between 1983 and 1990, there was no resistance to fluoroquinolones amongst *E. coli* isolates, however, 28% were found to be resistant in 1991 through 1993. This change corresponded to an increase in the rate of fluoroquinolone use from 1.4% of patients between 1983 and 1985 to 45% between 1991 and 1993 (62).

In another study done in Gondar, Ethiopia it was found that a high proportion of the common pathogens isolated were resistant to the frequently used antibiotics including tetracycline, chloramphenicol, sulphamethaxazole/trimethoprim and ampicillin (5). A Similar relationship has been reported in a study that was done in Sidamo, Ethiopia between 1985 and 1997, which showed that resistance pattern correlated with the commonly prescribed antibiotics (53). In a study done in Tanzania between 1978 and 1979, Maselle *et al* reported that 50-90% of all coliforms were resistant to ampicillin, tetracycline and sulphonamides which were the commonly used antimicrobials (63). Mhalu *et al* in a study done in Tanzania, also reported that excessive use of tetracycline during the cholera epidemic in the late 1970's contributed to a significant increase in the widespread resistance to the antimicrobial agent (64).

Other factors that favour development of antimicrobial resistance include a heavy burden of infectious diseases, large populations with rudimentary primary health care. Others are poor diagnostic services prompting health care workers to prescribe indiscriminately, poor sanitation and overcrowding, all of which tend to favour the rapid spread of resistant organisms (65,66).

Self–medication practice is very common in many parts of the developing countries (65). In a study done in Kenya, it was found that about 64% all antibiotics sold by the chemists were without prescriptions from a doctor (67). Due to lack of control, broad-spectrum antibiotics can be found being sold freely over the counter and even at open market places (48,65). Some pharmaceutical companies have vigorously been seeking markets for their products in areas that can least afford expensive drugs (48,65,68). Some antibiotics being sold are often of substandard qualities; these are therefore cheap and thus preferred by many people. (65).

Ignorance and poverty are other contributing factors such that some patients stop taking medication before completing recommended dose when they are feeling better or because they cannot afford the full dose (65,68). Resistant bacteria have been known to spread from one place to another through recent increases in air travel and globalisation (48,66). Widespread use of antibiotics in animal feeds, treatment of animal diseases, crop production and fish farming has also contributed to a new breeding ground for resistant microorganisms (66,69). The consequences of antimicrobial resistance may include limitation in therapeutic options, high costs of alternative drugs and an increase in morbidity and mortality (70). In the USA, the additional cost required to treat antibiotic resistant

bacteria was estimated to be between USD 4 to 5 million per year (71). The costs in the developing countries could be higher.

2.0 Statement of the problem

Pyogenic infections account for a significant proportion of bacterial infections. The antimicrobial susceptibility patterns of organisms causing pyogenic infections are dynamic and have been shown to vary with time, geographic area and are influenced by prescribing characteristics (72). Hence, there was a need to study the patterns and antimicrobial susceptibility of pus isolates so as to have the current knowledge. This new knoweledge can be used to predict the most probable causative organisms and also to predict the most effective antibiotics to be used before laboratory results are available. The knowledge can further be used to produce guidelines for the hospital and the nation in general on what antimicrobial agents should be used in pyogenic infections. In Tanzania, information on the antimicrobial susceptibility is either scanty or outdated due to absence of regular surveillance of susceptibility patterns of various pathogens. Therefore the results obtained from the proposed study would provide useful information to guide management of patients with bacterial infections.

3.0 Study Objectives:

3.1 Broad Objective

To determine the patterns and antimicrobial susceptibility of bacterial isolates causing pyogenic infections at the Muhimbili National Hospital, Dar es Salaam, Tanzania.

3.2 Specific Objectives:

1. To describe the patterns and causative organisms of superficial pyogenic infections at Muhimbili National Hospital.
2. To describe the demographic characteristics of patients suffering from different pyogenic infections at Muhimbili National Hospital.
3. To determine the antibiotic susceptibility patterns of bacterial isolates causing pyogenic infections at Muhimbili National Hospital.
4. To compare the antimicrobial susceptibility patterns of bacterial pathogens causing pyogenic infections among inpatients and outpatients at Muhimbili National Hospital.
5. To determine the relationship between antibiotics consumption and antibiotic susceptibility pattern in inpatients at Muhimbili National Hospital.

3.3 Hypotheses

1. There is no difference in the pattern of bacterial isolates causing pyogenic infections in relation to gender, age, patient status and antibiotic consumption at Muhimbili National hospital.
2. There has been no significant increase in proportion of pyogenic bacteria resistant to commonly used antimicrobial agents.

MEDICAL LIBRARY DAR ES SALAAM

4.0 Materials and methods

4.1 Study design

This was a cross sectional study, which was carried out over a period of one month from 1st to 31st of September, 1999 at the Muhimbili National Hospital, Dar es Salaam, Tanzania.

4.2 Setting

Muhimbili National Hospital is a tertiary health facility. It also serves as both the national referral hospital and university teaching hospital. The department of Microbiology and Immunology offers diagnostic services to patients in Dar es Salaam and the surrounding regions.

4.3 Collection of clinical data

The in-patients whose samples had been submitted to the Microbiology department were later on followed up in their respective ward to ascertain about their demographic information and inquire about history and nature of their infections. This information about antibiotic consumption for the in-patients was obtained from the yearly records available in the hospital pharmacy.

4.4 Sample collection

The study samples consisted of pus or pus swabs which were brought to the microbiology laboratory from patients with suspected pyogenic infections of the wounds, umbilical cord stumps, ears, post-surgical wounds, eyes, abscesses, burn injury, skin and traumatic wounds. The samples were collected by respective doctors as part of routine clinical management of patients admitted in medical, surgical and paediatric wards and from various outpatient clinics at the Centre. The specimen were inoculated on blood agar and MacConkey agar plates which were then incubate aerobically at 37°C for 24 hours. Anaerobic incubation was not done due to lack of reagents and facilities to achieve anaerobiasis. A total of 305 bacterial isolates were obtained on initial isolation from the specimens. On subculture, 248 isolates were recovered and 57 failed to grow. Of the remaining, 39 isolates were lost during transportation to Bergen, Norway leaving back 209 isolates for final identification.

4.5 Storage of bacterial isolates

The isolates were stored in nutrient agar slopes at room temperature for further identification and testing in Bergen, Norway. In Norway the samples were aliquoted into 2.5ml Nunc tubes and stored at -80°C until the time of identification and testing of antimicrobial susceptibility.

4.6 Bacterial identification

The samples were inoculated on blood agar and Lactose agar plates (Difco Laboratories, Becton Dickinson, France SA) followed by aerobic incubation at 37°C for 18-24 hours. The isolated colonies were identified based on characteristic colonial morphology, Gram stain appearance and standard biochemical tests as described in Cowan and Steel's manual for the identification of bacteria (73). Identification of difficult strains was performed using the API 20 NE for identification of non-enterobacteriaceae and API 20E for identification of enterobactericeae (BioMerieux SA, France).

4.7 Data on antimicrobial consumption

Data on antibiotic consumption was obtained from in-patient and hospital pharmacy records. Antibiotic consumption was presented as Daily Defined Doses (DDD) per patient days per 1000. The following DDDs were used: ampicillin 2g, Gentamicin 0.24g, cefuroxime 1g, chloramphenicol 3g, erythromycin 1g and ciprofloxacin 1g. Only six of the antibiotics tested for resistance were used in DDD calculation because the rest of the antibiotics were either not in stock in the hospital pharmacy or their DDD values were not available. Data on patient hospital days were obtained from the Medical Records Unit of the Muhimbili National Hospital. The DDD and the patient hospital

MEDICAL LIBRARY

days were used to calculate antimicrobial consumption for the in-patients.

4.8 Antibiotic susceptibility testing

The National Committee for Clinical Laboratory Standards (NCCLS) disk diffusion method was used for antimicrobial susceptibility testing using Mueller-Hinton agar medium (Difco Laboratories, Becton Dickinson, France, SA) (74). Barium sulphate 0.5 McFarland was used as a turbidity standard for the inoculum (74). The 0.5 McFarland standard solution was then placed in 4-6 mL aliquot tubes identical to those used in growing or diluting the bacterial inoculum. The tubes were then stored in the dark at room temperature. The turbidity standards were shaken and inspected for uniform turbidity before each use. Three to five well-isolated colonies of similar appearance were selected from the agar plate culture. The top of each colony was touched with a loop and the growth transferred into a tube containing 4-5 mL tryptic soya broth and incubated at 35°C for 2-6 hours. Adjustment was made by diluting the broth culture with sterile broth or sterile saline to obtain turbidity comparable to that of 0.5 McFarland. The comparison was done visually under adequate light. The well-dried surfaces of Mueller-Hinton (Difco laboratories, Becton Dickinson, France S.A) agar plates were inoculated within 15 minutes of adjusting the inoculum. A

sterile cotton swab was dipped into the inoculum, rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculum. The whole sterile agar surface was inoculated. This procedure was repeated twice, rotating the plate approximately 60° each time to ensure an even distribution of the inoculum. As a final step, the rim of the agar was swabbed. To dry the plates, the lids were left open for 3-5 minutes at room temperature to allow excess surface moisture to be absorbed. A mechanical dispenser was used to place antibiotic discs (AB Biodisk, Solna, Sweden). This allowed the discs to be evenly distributed so that they were no closer than 24mm from center to center. A maximum of six discs were used on one 90mm diameter petri dish plate. The plates were then incubated within 15 minutes of applying discs. The plates were incubated at 35°C for 16-18 hours. The control organisms were *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 for the gram positives, gram negatives and pseudomonads respectively (74).

After an over-night incubation the diameter in millimeters of each inhibition zone (including the diameter of the disc) were measured and recorded. The measurements were made with the aid of measuring calipers. The zone margins were taken as the area showing no obvious, visible growth that can be detected with the

unaided eye. With *Proteus* spp, the thin veil of swarming in an otherwise obvious zone of inhibition was ignored. Interpretation was made by comparison of inhibition zones with published tables of critical zone diameters. The results were reported as susceptible, intermediate, or resistant to the agents that had been tested (74).

S. aureus and coagulase negative staphylococci that were found to be resistant or intermediately sensitive to methicillin and vancomycin were re-tested with E-test (AB Biodisk, Solna, Sweden). Colonies of the organisms to be tested were taken from 24-48 hours plates and placed in 0.85% saline, homogenized to achieve turbidity equivalent to 0.5 McFarland. A dry sterile swab was then dipped into the inoculum, excess fluid was removed by pressing to the side of the tube. A well-dried surface of Mueller Hinton agar with 2% NaCl was swabbed evenly in three directions. The surface was allowed to dry for about 10-15 minutes. An oxacillin strip was then applied to the agar surface with the MIC scale facing upwards using an E-test applicator. The dried agar plate was then incubated at 35°C for 24 and 48 hours for *S. aureus* and coagulase negative staphylococci respectively. The MIC value was read at the place where the ellipse intersects with the MIC scale. A value less than or equal to 2 was considered sensitive whereas a value greater than or equal to 4 was considered to be resistant. When testing for vancomycin resistance among

staphylococci, a Brain Heart Infusion broth was used to prepare inoculum. A heavy inoculum was used to achieve a turbidity of 2 McFarland. An amount equivalent to 0.2ml of the suspension was then poured onto a well-dried surface of a 90mm Brain Heart Infusion agar plate and streaked evenly. After being left to dry, a vancomycin E-test strip was applied and incubated at 35°C for 48 hours. A value below or equal to 4 was considered to be sensitive whereas a value greater than or equal to 32 was regarded to be resistant (75).

4.9 Choice of antibiotic discs for sensitivity testing

First line antimicrobial agents are those that are commonly prescribed for bacterial infections. Second line drugs are used when there is no response or there is high resistance to the first line antimicrobial agents treatment.

Organisms	**First line**	**Second line**
Gram-positive	Penicillin	Gentamicin
	Oxacillin	Vancomycin
	Trimethoprim/	Clindamycin
	Sulphamethaxazole	Cefuroxime
	Ampicillin	
	Erythromycin	
	Tetracycline	
Gram- negative	Ampicillin	Amikacin
Bacteria	Gentamicin	Cefuroxime
	Trimethoprim/	Tobramycin
	sulphamethaxazole	Norfloxacin
	Tetracycline	Ceftazidime
	Augumentin	Ciprofloxacin
	Chloramphenicol	
P.aeruginosa	Ceftazidime	Imipenem
and other	Gentamicin	
pseudomonads	Tobramycin	
	Amikacin	
	Ciprofloxacin	

4.10 Statistical analysis

The results were analysed by SPSS for Windows version 9.0 (76). Comparison between proportions was estimated by Chi-square test. Fisher's exact test was used when the grand total was less than twenty (n=20) or when the expected frequency was less than five (5). Intermediate sensitive isolates were considered as fully sensitive during analysis. Double sided p-value less than 0.05 was used to indicate statistical significance.

4.11 Ethical clearance

The ethical clearance was obtained from the Higher Degree, Research and Publication Committee, of the Muhimbili University College of Health Sciences, Dar es Salaam, Tanzania.

4.12 Limitations of the study

1. The time period for data and specimen collection was short, hence, certain rare types of specimen were not adequate.
2. Due to the small number of the isolates, in some cases statistical tests were not done.
3. Calculation and analysis of antimicrobial consumption in relation to resistance pattern was limited to six antimicrobial agents only because the DDD values of other antimicrobial agents were not available or the antimicrobial agents were not in stock.
4. The calculation of DDD/patient days/1000 was done with assumption that all in-patients received antibiotics.
5. Information collected on antimicrobial use in the in-patients was not used in the analysis because it was inadequate, this was due to the fact that some patients did not know what antibiotics they were receiving and their hospital files or treatment charts were not properly recorded.

5.0 Results

Pus and pus swabs were collected from 145 patients, from which 209 bacterial isolates were recovered and identified. There were 78(53.8%) male and 67(46.2%) female patients. A total of 36 patients had mixed infections that yielded more than one bacterial isolate. The mean (median) age of the patients was 20.7 (17) years (range 1 day to 89 years). Table 5.1 shows the age distribution of the subjects in the study.

Table 5.1: Age distribution of patients with pyogenic infections

AGE	No. of patients	Percentage (%)
Neonates (1d-1m‡)	35	24.1
Children (2m-18y‡)	39	26.9
Adults (> 19y‡)	71	49
Total	145	100

‡D=Days, M=Months and Y=Years

Out of the 209 isolates that were identified, *S. aureus* was the most prevalent organisms comprising of 47 isolates (22.5%), followed by coagulase negative *Staphylococcus* spp accounting for 27 isolates (12.9%). *P. mirabilis* and *P. aeruginosa* were also common comprising of 26 isolates (12.4%) and 22 isolates (10.5%) respectively (Table5.2).

Table 5.2: Prevalence of bacteria species isolated from pyogenic infections

Organisms	No.of isolates	%
Staphylococcus aureus	47	22.5
Coagulase negative staphylococci*	27	12.9
Proteus mirabilis	26	12.4
Pseudomonas aeruginosa	22	10.5
Escherichia coli	18	8.6
Enterobacter spp	12	5.7
Klebsiella ozaenae	10	4.8
Providencia spp	10	4.8
Enterococcus spp	9	4.3
Corynebacterium spp	6	2.9
Alcaligenes faecalis	5	2.4
Morganella morganii	4	1.9
Acinetobacter baumannii	2	1.0
Moraxella catarrhalis	2	1.0
Citrobacter spp	2	1.0
Pseudomonas spp	2	1.0
Streptococcus pneumoniae	2	1.0
Micrococcus spp	1	0.5
Ochrobactrum anthropi	1	0.5
Proteus vulgaris	1	0.5
Total	209	100.0

*Coagulase negative staphylococci comprised of *S. saprophyticus*, *S. epidermidis* and other coagulase negative staphylococci.

Table 5.3: Common organisms in relation to source of specimen

Organisms	Wound	Umbilical cord	Ear	Eye	Burn injury	Post-surgical wounds	Abscesses	Others	Total
S. aureus	11	14	3	1	5	4	6	3	**47**
*CNS**	6	6	3	7	0	1	1	3	**27**
P.mirabilis	10	5	4	1	1	2	1	2	**26**
P. aeruginosa	8	4	5	1	3	0	0	1	**22**
E. coli	4	8	2	1	0	1	0	2	**18**
Enterobacter spp	1	6	2	0	0	2	1	0	**12**
K. ozaenae	4	2	1	1	0	1	1	0	**10**
Providencia spp	2	0	7	0	0	1	0	0	**10**
Enterococcus spp	2	3	0	0	2	1	1	0	**9**
Corynebact. spp**	1	0	1	2	0	1	1	0	**6**
A. faecalis	0	0	2	0	0	1	1	1	**5**
Morganella spp	2	0	0	0	0	2	0	0	**4**
A. baumannii	0	2	0	0	0	0	0	0	**2**
S. pneumoniae	0	0	1	0	1	0	0	0	**2**
M. catarrhalis	2	0	0	0	0	0	0	0	**2**
Citrobacter spp	1	0	0	0	0	0	0	1	**2**
Pseudomonas spp	0	0	1	1	0	0	0	0	**2**
Micrococcus spp	0	0	0	1	0	0	0	0	**1**
O. anthropi	1	0	0	0	0	0	0	0	**1**
P. vulgaris	0	0	1	0	0	0	0	0	**1**
Total	**55**	**50**	**33**	**16**	**12**	**17**	**13**	**13**	**209**

*Coagulase negative staphylococci **Corynebacterium spp

S. aureus was the most prevalent organism found in wound infections accounting for 11 isolates (20.0%), followed by *P. mirabilis, P. aeruginosa* and coagulase negative staphylococci consisting of 10 isolates (18.2%), 8 isolates (14.6%) and 6 isolates (10.9%) respectively (Table 5.3).

S. aureus was the most prevalent organism in post-surgical wound infections comprising of 4 isolates (23.5%), followed by *M. morganii,*

Enterobacter spp and *P. mirabilis* each with 2 isolates (11.8 %). Gram-negative rods were the commonest isolates in the ear discharges with *Providencia* spp, *P. aeruginosa* and *P. mirabilis* accounting for 7 (21.2%), 5 (15.2%) and 4 (12.1%) of all isolates respectively. *S. saprophyticus* and other coagulase negative staphylococci were common isolates in eye infections with 4 (25%) and 3 (18.8%) isolates respectively.

In burn injury, *S. aureus* and *P. aeruginosa* were found to be the commonest organisms with 5 (41.7%) and 3 (25%) of all isolates respectively. *S. aureus* was the commonest isolate in the umbilical cord stump infection followed by *E. coli*, coagulase negative staphylococci and *Enterobacter* spp accounting for 14 (28%), 8 (16%), 6(12%) and 6 (12%) of all isolates respectively. In abscesses, *S. aureus* was the prevalent organisms accounting for 6 (46.2%).

Table 5.4: The frequency of the bacterial isolates in relation to age*§

Age	GPC(%)[1]	GNR(%)[2]	PSE(%)[3]	Others(%)	Total(%)
Neonates(1d-1m)**	31(46.3)	28(41.8)	6 (8.9)	2(3.0)	67(100)
Children(2m-18y)**	21(42.0)	20(40.0)	8(16.0)	1(2.0)	50(100)
Adults(>19y)**	34(37.0)	37(40.2)	16(17.4)	5(5.4)	92(100)
Total	86	85	30	8	209

*Figures in brackets represent percentage of each group of organisms out of the total number of isolates in each age group.

**D=Days, M=Months and Y=Years

[1]GPC= Gram positive cocci, [2]GNR= Gram negative rods, [3]PSE= Pseudomonads

§ All differences were not statistically significant.

Table 5.4 shows the distribution of common microorganisms in relation to the age of the patients. The Gram-positive cocci and Gram-negative rods were evenly distributed across all age groups (p-value=0.495). Pseudomonads were found more commonly in adults than the other age groups (p-value=0.308). There were no significant differences in the distribution of the pathogens in various age groups (p-value >0.05).

Table 5.5 shows the distribution of organisms in relation to gender. There was no significant difference in the distribution of the Gram-positive cocci in males and females (p-value=0.181). There were more Gram-negative rods in males than in females but the difference was not statistically significant (p-value=0.605). Pseudomonads were found to be twice as much more common in males as compared to the females however the difference was not statistically significant (p-value=0.184).

Table 5.5: The frequency of the bacterial isolates in relation to gender*§

Gender	GPC[1]	GNR[2]	PSE[3]	Others	Total
Males	43(37.1)	49(42.2)	20(17.3)	4(3.4)	116(100)
Females	43(46.2)	36(38.7)	10(10.8)	4(4.3)	93(100)
Total	86	85	30	8	209

*Figures in brackets represent percentage of each group of organisms out of the total number of isolates in each gender group.

[1]GPC= Gram positive cocci, [2]GNR= Gram negative rods, [3]PSE= Pseudomonads

§ All differences were not statistically significant.

Table 5.6 shows the distribution of organisms in relation to patient status. *S. aureus, P. mirabilis, P. aeruginosa, E. coli, Enterobacter spp, K. ozaenae* were found more commonly in in-patients than in out-patients although the differences were not statistically significant (p-value>0.05). *Providencia* spp was found more commonly in out-patients than in the in-patients but the difference was not statistically significant (p-value>0.05).

Table 5.6: The frequency of the bacterial isolates in relation to patient status§

Organisms	Out-patients		In-patients		Total
	n	%	n	%	
S. aureus	13	18.1	34	24.8	47
CNS*	13	18.1	14	10.2	27
P. mirabilis	6	8.3	20	14.6	26
P. aeruginosa	8	11.1	14	10.2	22
E. coli	3	4.2	15	10.9	18
Enterobacter spp	4	5.6	8	5.8	12
K.ozaenae	3	4.2	7	5.1	10
Providencia spp	7	9.7	3	2.2	10
Enterococcus spp	4	5.6	5	3.6	9
Corynebacterium spp	2	2.8	4	2.9	6
A. faecalis	2	2.8	3	2.2	5
M. morganii	0	0.0	4	2.9	4
A. baumannii	0	0.0	2	1.5	2
M. catarrhalis	0	0.0	2	1.5	2
Pseudomonas spp	2	2.8	0	0.0	2
Citrobacter spp	1	1.4	1	0.7	2
S. pneumoniae	1	1.4	1	0.7	2
Micrococcus spp	1	1.4	0	0.0	1
O. anthropi	1	1.4	0	0.0	1
P. vulgaris	1	1.4	0	0.0	1
Total	72	100.0	137	100.0	209

*CNS=Coagulase negative staphylococci

§ All differences were not statistically significant.

Table 5.7: Antibiotic resistance pattern of Gram positive bacteria*†

Organisms	No.of isolates	PEN	AMP	OXA	SXT	ERY	DOX	GEN	CLI	CXM
S.aureus	47	87.2	87.2	2.1	34	34	29.8	2.1	2.1	0
CNS‡	27	81.5	51.9	11.1	33.3	44.4	44.4	7.4	0	11.1
Corynebacterium spp	6	33.3	16.7	50	50	33.3	0	16.7	33.3	0
Enterococcus spp	9	11.1	11.1	100	22.2	44.4	88.9	44.4	88.9	88.9
S.pneumoniae	2	0	0	0	0	0	0	0	0	0
Micrococcus spp	1	100	0	0	100	0	100	0	0	0

*Figures given represent percentage of resistance.

PEN=penicillin, **AMP**=ampicillin, **OXA**=methicillin, **CXM**=cefuroxime
SXT=sulphamethoxazole/trimethoprim, **ERY**=erythromycin,
DOX=doxycycline, **GEN**=gentamicin, **VAN**=vancomycin, **CLI**=clindamycin

‡CNS=Coagulase negative staphylococci

†All the Gram positive cocci were not resistant to vancomycin.

Table 5.7 shows the antimicrobial resistance pattern of the bacterial isolates. Forty-seven *S. aureus* were isolated of which 87% were resistant to both penicillin and ampicillin, while 34% were resistant to erythromycin and sulphamethoxazole/trimethoprim and 2.1% were resistant to methicillin. None of *S. aureus* isolates were resistant to cefuroxime or vancomycin.

Among CNS, 81.5%, 51.9%, 44.4%, 44.4% were found to be resistant to penicillin, ampicillin, erythromycin and doxycycline respectively. Methicillin resistance among the coagulase negative staphylococci

was 11.1%. The two *S. pneumoniae* isolates were sensitive to all antimicrobial tested. None of the enterococci showed resistance to vancomycin, however 88.9% of the isolates were resistant to doxycycline, clindamycin and cefuroxime.

Table 5.8 shows resistance pattern of the gram-negative organisms. Many Gram-negative organisms showed high resistance to many antimicrobials. More than 40% of all isolates were resistant to ampicillin. *Proteus* spp isolates were resistant to sulphamethoxazole/ trimethoprim (40.7%), ampicillin (55.6%) and doxycycline (92.6%). *Proteus* spp was the only organism that showed resistance to gentamicin (11.1%). Among the gram-negative rods 61.1%, 83.3% and 70% of *E. coli*, *Enterobacter* spp and *Klebsiella* spp were resistant to ampicillin respectively. Apart from *Citrobacter* spp, the rest of gram-negative rods tested showed high resistance to doxycycline ranging from 40% in *Klebsiella* spp to 100% in *Providencia* spp. *Klebsiella* spp was the only organism to have shown resistance to ciprofloxacin with 10% of the isolates being resistant to this antimicrobial. High resistance was also noted for amoxycillin/clavulanic acid ranging from 5.6% in *E. coli* to 100% in *Providencia* spp. No resistance was noted for amikacin, tobramycin, norfloxacin and ceftazidime among the Gram-negative rods.

LIBRARY MED.CAL

Table 5.8: Antibiotic resistance pattern of Gram negative bacteria*†

Organisms	No. of isolates	SXT	AMP	DOX	GEN	CXM	AMC	CHL	AMK	NOR	CAZ	CIP
Proteus spp	27	40.7	55.6	92.6	11.1	14.8	14.8	48.1	0	0	0	0
E. coli	18	27.8	61.1	50	0	0	5.6	16.7	0	0	0	0
Enterobacter spp	12	8.3	83.3	41.7	0	16.7	41.7	8.3	0	0	0	0
Klebsiella spp	10	50	70	40	0	0	10	30	0	0	0	10
Providencia spp	10	0	40	100	0	0	100	70	0	0	0	0
Morganella spp	4	50	75	75	0	50	25	50	0	0	0	0
Citrobacter spp	2	0	100	0	0	0	50	0	0	0	0	0

*Figures given represent percentage of resistance.

SXT=trimethoprim/sulphamethoxazole, **AMP**=ampicillin, **DOX**=doxycycline, **GEN**=gentamicin, **CXM**=cefuroxime, **AMC**=amoxycillin/clavulanic acid, **CHL**=chloramphenicol, **AMK**=amikacin, **TOB**=tobramycin, **NOR**=norfloxacin, **CAZ**=ceftazidime, **CIP**=ciprofloxacin

*†All the isolates were sensitive to tobramycin.

Table 5.9:Antibiotic resistance pattern of Pseudomonads *

Organisms	No. of isolates	GEN	AMK	TOB	CAZ	CIP	IMP
Pseudomonas spp	24	0	0	0	0	0	0
Alcaligenes faecalis	5	0	0	20	0	20	0

*Figures given represent percentage of resistance.

GEN=gentamicin, **AMK**=amikacin, **TOB**=tobramycin, **CAZ**=ceftazidime, **CIP**=ciprofloxacin, **IMP**=imipenem.

Table 5.9 shows the resistance pattern of pseudomonads. All of the 24 *Pseudomonas* spp isolates were sensitive to all antimicrobials agents tested. Among the *Alcaligenes faecalis* isolates 20% were resistant to both ciprofloxacin and tobramycin.

Table 5.10 shows the antimicrobial resistance pattern of staphylococci in in-patients and out-patients. Both in-patient and out-patient *S. aureus* isolates showed similar resistance to penicillin and ampicillin (p-value=0.876). Methicillin and gentamicin resistant isolates were found in in-patients only while clindamycin resistant isolates were found in out-patients only (p-value>0.05). More Doxycycline resistance was noted among isolates from out-patients than from the in-patients (p-value=0.24). Coagulase negative staphylococci resistant to methicillin and cefuroxime were found from in-patients only (p-value=0.05). The resistance patterns of CNS to other antimicrobials agents were found to be nearly the same in both groups. Despite the above findings, there were no significant differences in the resistance pattern of isolates from in-patients and out-patients (p-value > 0.05).

Table 5.10: Antimicrobial resistance pattern of Staphylococci spp in in-patients and out-patients*§

Organisms	No	PEN	AMP	OXA	SXT	ERY	VAN	CLI	CXM	DOX	GEN
S.aureus											
In-patients	34	88.2	88.2	2.9	32.4	35.3	0	0	0	23.5	2.9
Out-patients	13	84.6	84.6	0	38.5	30.8	0	7.7	0	46.2	0
CNS											
In-patients	14	78.6	50	21.4	28.6	50	0	0	21.4	42.9	7.1
Out-patients	13	84.6	53.8	0	38.5	38.5	0	0	0	46.2	7.7

*Figures given represent percentage of resistance.

§ All differences were not statistically significant.

Table 5:11 show the resistance pattern of Gram-negative rods among the in-patient and out-patient isolates. Regarding *Proteus* spp, the resistance patterns of most antimicrobials were similar in the two groups, except for gentamicin and amoxycillin/clavulanic acid, where resistant isolates were only those from in-patients. Sixty percent of the *Enterobacter* spp isolates from the outpatients were resistant to doxycycline compared to 28.6% of the in-patient isolates. None of the Gram negative bacteria were resistant to amikacin, tobramycin, norfloxacin and ceftazidime. Significance testing was not done due to the small number of isolates in each group.

Table 5.11: Antimicrobial resistance pattern of gram negative bacteria in in-patients and out-patients*§†

Organisms	(No)	SXT	AMP	DOX	GEN	CXM	AMC	CHL	AMK	CAZ	CIP
***Proteus* spp**											
In-patients	20	40	55	90	15	10	20	50	0	0	0
Out-patients	7	50	50	100	0	16.7	0	50	0	0	0
E.coli											
In-patients	15	33.3	60	53.3	0	0	0	20	0	0	0
Out-patients	3	0	66.7	33.3	0	0	33.3	0	0	0	0
***Enterobacter* spp**											
In-patients	7	0	85.7	28.6	0	14.3	42.9	0	0	0	0
Out-patients	5	20	80	60	0	20	40	20	0	0	0
***Klebsiella* spp**											
In-patients	7	42.9	71.4	42.9	0	0	0	28.6	0	0	14.3
Out-patients	3	66.7	66.7	33.3	0	0	33.3	33.3	0	0	0
***Providencia* spp**											
In-patients	3	0	66.7	100	0	0	100	66.7	0	0	0
Out-patients	7	0	28.6	100	0	0	100	71.4	0	0	0

*Figures given represent percentage of resistant isolates.

†All the isolates were sensitive to tobramycin and norfloxacin.

§All the differences were not statistically significant.

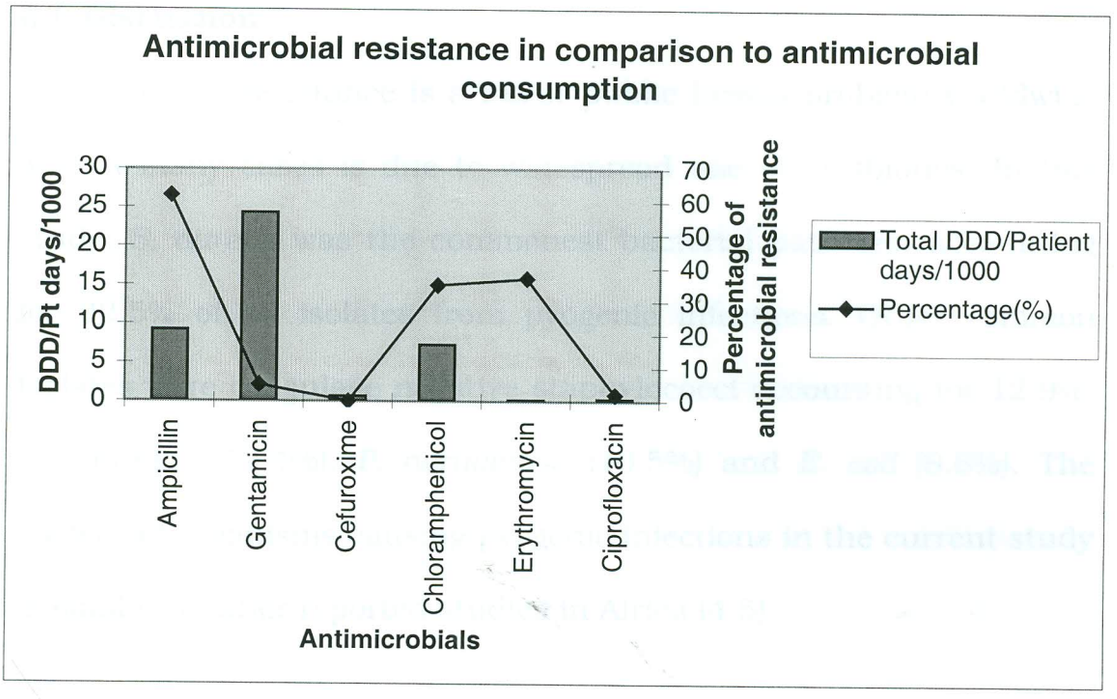


Figure I: Shows antimicrobial consumption in the hospital for the year 1999. It was calculated as DDD/patient days/1000. It was compared to the antimicrobial resistance in percentage.

Figure I show antimicrobial consumption in relation to resistance pattern. There was high consumption of ampicillin, gentamicin and chloramphenicol and low consumption of cefuroxime and ciprofloxacin. The high consumption of ampicillin and chloramphenicol was associated with high levels of antimicrobial resistance whereas for gentamicin and erythromycin there was no association noted.

6.0 Discussion

Antimicrobial resistance is a major public health problem worldwide and in many cases is due to widespread use of antibiotics. In this study, *S. aureus* was the commonest bacterial pathogen accounting for 22.5% of all isolates from pyogenic infections. Other common isolates were coagulase negative staphylococci accounting for 12.9%, *P. mirabilis* (12.4%), *P. aeruginosa* (10.5%) and *E. coli* (8.6%). The pattern of organisms causing pyogenic infections in the current study is similar to other reported studies in Africa (4,5).

In the present study, *S. aureus* was also the most predominant organism in wounds, post-surgical wounds, cord sepsis, burn injury and abscesses. In a study done between 1973 and 1987 by Brook *et al*, they found *S. aureus* to be the most predominant organism in wound infection followed by *S. pyogenes* and *E. coli* (10). A review by Bowler in 1998 showed *S. aureus* and *P. aeruginosa* to be the most important wound pathogens (37). Other prevalent isolates in wound infection in the present study, included *P. mirabilis*, *P. aeruginosa* and coagulase negative staphylococci. Many reports have shown *S. aureus* to be the most prevalent in post–surgical wound infections. Other organisms that have been found in post-surgical wounds infection include coagulase negative staphylococci, enterococci, *E. coli* and *P. aeruginosa* (14,16,77). In burn injury *S. aureus* has been

reported to be the most prevalent organism together with *P. aeruginosa* and *Klebsiella* spp (30,78). Studies have shown *S. pneumoniae, H. influenza* and *Moraxella catarrhalis* to be the main pathogens in otitis media (16,79). In the present study, *Providencia* spp, *P. aeruginosa, P. mirabilis, S. aureus* and coagulase negative staphylococci were the most prevalent organisms. Due to technical problems the bacterial isolates in the present study were left for about six months without being sub-cultured. Given the delicate and fastidious nature of *S. pneumoniae* and *Haemophilus* spp, it is possible that these organisms might have died while waiting to process the samples. A total of 57 bacterial isolates could not be recovered and 39 bacterial isolates got lost.

Methicillin resistance among staphylococci is widespread in some countries. In the United States of America, a study done in hospitals showed that methicillin resistance among *S. aureus* increased from 2.4% in 1975 to 29% in 1991(80). In Western Australia, Riley *et al* reported that there was a rapid increase in the proportion of MRSA from about 6% in 1989 to about 58% in 1991 (81). In Europe a more diverse picture is seen, Sweden and Denmark have reported the lowest methicillin resistance rates of 0.1% and 0.3% respectively while higher rates of resistance have been reported in Spain, France and Italy with 30.3%, 33.6% and 34.4% respectively (82). The pattern

of methicillin resistance among *S. aureus* is also varied in the developing countries, where figures as high as 66.4% in Senegal (83), 40% in Kenya (72) and as low as 4% in Zimbabwe (84) have been reported. However, methicillin resistant *S. aureus* seems not to be a big problem in Dar es Salaam, Tanzania, but it is rising from a figure of 0.4% in 1998 to the current figure of 2.1% in the current study (57).

It is encouraging to note that in the current study, there was no vancomycin resistance noted among the *S. aureus* isolated. This is similar to a study reported by Voss *et al* which included 43 laboratories in 10 European countries in 1994 and another study done by de Neeling *et al* in the Netherland in 1999, in which they reported absence of resistance of *S. aureus* to vancomycin (82,85). Similarly, absence of vancomycin resistance has been reported in some developing countries. In a study done by Geyyid *et al* in Ethiopia between 1987-88 and another done by Orrett in Trinidad between 1997-98 they both found lack of resistance to vancomycin among *S. aureus* isolates (86,87). However, cases of vancomycin intermediate resistant strains of *S. aureus* have been reported in Japan, USA and France among methicillin resistant *S. aureus* (88-92).

MEDICAL ... DAR ES SALAAM

In the current study, 87% of the *S. aureus* isolates were resistant to penicillin. These results are comparable to 90%, 78% and 90.6% reported by Maselle *et al* in 1978, 1979 and 1982 respectively (39). Urassa *et al* in 1998 reported a penicillin resistance of 93.5% (57). These findings show that penicillin resistance at Muhimbili National Hospital has remained persistently high over the years. However, this problem has also been found in other countries, for example, penicillin resistance in studies done in Zimbabwe, The Netherlands and Ethiopia were reported to be 69%, 78% and 83.1% respectively (84-86).

In Tanzania, earlier studies showed that *S. aureus* resistance to erythromycin was12.8% in 1978, 2% in 1979 and 3.3% in 1982 (39). By 1998 it had risen to 32% according to findings by Urassa *et al* in 1998. In the present study the resistance to erythromycin was 34%. This upward trend could be attributed to wide spread use of erythromycin as alternative to ampicillin in treatment of Gram-positive bacterial infections. *S. aureus* resistance to tetracycline was reported to be 44%, 57.1%, 74.4% and 61% in 1978,1979,1982 and 1998 respectively (39,57) in contrast to 29.8% in the present study. Following widespread use of tetracycline in treating cholera cases during the epidemic in mid 1970s, a sharp increase in the resistance to this antimicrobial was reported (64). The use of tetracycline has

decline in recent years, probably leading to improved sensitivity of *S. aureus* to this antimicrobial.

Coagulase negative staphylococci (CNS) which are normal microflora of the skin and mucous membranes are increasingly being implicated in a wide range of infections including post-operative wound infections, after cardiac surgery and use indwelling devices like central venous catheters, peritoneal dialysis catheters and shunts (93). Bitkover *et al* reported that CNS constituted between 33 and 62.5% of wound infection after cardiac surgery (94) while Yalcin *et al* reported a prevalence of 21.7% in post-operative wound infection cases in Turkey (77). CNS are of clinical importance due to their high antimicrobial resistance. Jarløv *et al* reported that 56% of coagulase negative staphylococci were resistant to methicillin, while 86%, 51% and 40% were resistant to penicillin, gentamicin and erythromycin respectively (95). In the present study resistance to methicillin among coagulase negative staphylococci was 11% while that to penicillin, gentamicin and erythromycin were 82%, 7.4% and 44.4% respectively. Similar findings were also observed by de Neeling *et al* in 1998, in a study looking at resistance pattern among staphylococci in the Netherlands, where they found out that CNS were more resistant than *S. aureus* to methicillin and other antimicrobial agents (85). It is encouraging to note that none of the CNS in the current study was

resistant to vancomycin and clindamycin. It was not the intention to ascertain the pathogenic role of CNS in the present study, but because it is a potential opportunistic pathogen it was important to determine its resistance pattern.

Enterococci are increasingly becoming important nosocomial infection agents (96). In the United States they have been reported to account for about 10% of nosocomial infections (96). *Enterococcus faecalis* has been found to account for about 80% of human enterococcal infections while *E. faecium* is found in the rest of infections, although the latter isolates are usually more resistant to many antimicrobial agents. Other types of enterococci rarely cause infections (97). It has also been reported that infections caused by vancomycin resistant enterococci (VRE) increased 20-fold from 0.3% to 7.9% between 1989 and 1993 in the United States (97). In the present study, Enterococci showed significant resistance to clindamycin (88.9%), doxycycline (88.9%), gentamycin (44.4%) and erythromycin (44.4%). Enterococci are known to be inherently resistant to many antimicrobial agents, including acquired resistance to aminoglycosides and glycopeptides (97,98). As it was in the case of CNS there was no resistance to vancomycin noted in the present study. Studies in the US have shown high vancomycin and ampicillin resistance among enterococci (97).

Gram-negative bacteria including *P. mirabilis, P. aeruginosa, E. coli, Enterobacter* spp, *Klebsiella* spp and *Providencia* spp were among the commonest isolates in the present study. Gram-negative bacteria have been reported to be more resistant to common antimicrobials (99). Many bacteria showed high resistance to commonly used antimicrobials (99,100). Between 1978-9 there was no reported gentamicin resistance among the *Proteus* species in Tanzania (63). In the present study the resistance rate of *Proteus* spp to gentamicin was 11%. There was a high proportion of bacterial isolates resistant to ampicillin, tetraxycyline, chloramphenicol, trimethoprim/ sulphamethoxazole and amoxycillin / clavulanic acid.

P. aeruginosa has become increasingly resistant to many first line antibiotics as well as to third generation cephalosporins (101,102). Studies done in Intensive Care Units (ICUs) in Europe and the United States between 1990-91 reported *P. aeruginosa* resistance to ceftazidime ranging between 12-16% and those of imipenem, tobramycin and ciprofloxacin being 10-22%, 9-36% and 9-32% respectively (103). In Ethiopia the resistance of *P. aeruginosa* to gentamicin has been reported to be between 5 and 11% (5,53). A study done in Kenya reported a pseudomonas resistance of 43.8% to gentamicin with resistance to aztreonam, amikacin and ceftazidime being 10%, 2.9% and 5.9% respectively (72). In a study done in

Tanzania by Maselle *et al* in 1980 (63), it was reported that all pseudomonas isolates were sensitive to gentamicin which is in agreement with the present study where none of the isolates were resistant to gentamicin, amikacin, tobramycin, ceftazidime, ciprofloxacin and imipenem. Reasons for these findings are unclear, but could reflect low utilization of intramuscular gentamicin by the general population as opposed to many antimicrobials that are taken orally and easily dispensed. The other antimicrobials tested are not usually prescribed and in most cases are very expensive.

In the present study there was no relationship between the distribution of the organisms with age. This is in agreement with Seppala *et al* who, while studying the factors associated with erythromycin resistance to Group A streptococci, found out that there was no correlation between the ages of the patients and group A streptococci erythromycin resistance among the pus isolates (104). Likewise, isolates in both the male and female groups in the present study were found to be similar in distribution. There was no difference in the distribution of organisms and their antimicrobial resistance patterns among the in-patients and out-patients. The small sample size could have played a role. A larger study is required to ascertain this fact.

In the current study only two *S. pneumoniae* and no other streptococci were isolated and identified. This could be due to the delicate nature of the organisms. They might have died during transportation as the bacterial isolates had stayed for almost six month without sub-culturing from the time of initial isolation to the time of final identification. However, the two isolates of *S. pneumoniae* were sensitive to all antimicrobial agents tested. Previous studies from Tanzania showed a decline in the resistance pattern of these organisms. In 1978, 26.3% of *S. pneumoniae* were resistant to penicillin, but decreased to 7.7% in 1979 and there was no resistance detected in 1982 (39). This differs from the pattern reported from a study done in France by the National Reference Center in 1997 where it was found that between 1987 and 1997 the resistance to penicillin G had risen from 3.8% to 48% (105). Resistance of *S. pneumoniae* in the United States has increased dramatically over a period of time. It was reported that before 1990 most isolates were susceptible to a number of antimicrobial agents. However, the situation started to change in early 1990s. A study done in 1998 in the United States showed a penicillin resistance of 29.5% (106). The increasing resistance is attributed to transformation and mutations in the genes coding for penicillin binding proteins. This could be due to pressure from antibiotic use (107).

In the current study, the antimicrobial resistance pattern had some correlation with commonly used antibiotics. Ampicillin was one of the most consumed antimicrobial and it had a resistance rate of 87.2% among S. *aureus* and 40-100% among the Gram negative isolates in the present study. chloramphenicol has also been widely used to treat cases suspected to have typhoid fever. This might have led to widespread use of the antimicrobial and consequently its increasing resistance. In the present study the resistance to chloramphenicol was low among the Enterobacter spp and *E.coli* with 8.3% and 16.7% respectively. However, *Proteus* spp, *Klebsiella* spp and *Providencia* spp showed chloramphenicol resistance of 48.1%, 30% and 70% respectively. Despite a high consumption of gentamicin, its resistance among S. *aureus* was only 2.1%. Among the Gram-negative isolates only *Proteus* spp were resistant to gentamicin with 11.1% of the isolates tested being resistant to this antimicrobial. This could be explained by the fact that gentamicin being an injectable antimicrobial is not easily abused.

It is expected that increased use of antibiotics leads to increased resistance, but this relationship is not necessarily maintained for all antibiotics and organisms. When comparing CNS resistance in relation to antibiotic use in Denmark between 1995-96, Jarløv *et al* reported that aminoglycosides and gentamicin resistances were

related to antibiotic use. There was no association found for rifampicin, fusidic acid, clindamycin, tetracycline, microlides and erythromycin (95). Monsen *et al* reported similar findings when studying antibiotic susceptibility in relation to consumption among staphylococci in blood culture in Sweden. He reported a positive association between use and resistance of quinolones, oxacillin, trimethoprim/sulphamethaxazole and β-lactamase antibiotics, but there was no relation between consumption and resistance in aminoglycosides, clindamycin or fusidic acid (108).

In the present study there was an association between consumption and resistance in ampicillin and chloramphenicol, but there was no association noted for gentamicin and erythromycin. Lack of such association could be due to confounding factors related to therapeutic procedures like dosage, compliance and duration of treatment, types of patients and patterns of infectious diseases (61,109).

Rate of antibiotic consumption in the hospital was calculated using DDD/patient days/1000 with assumption that all inpatients did receive antimicrobials. Due to poor prescription records it was not possible to identify those who received antibiotics as treatment and those who received as prophylaxis. One of the weaknesses of this study was the data collected regarding antibiotic consumption. It

must be noted that due to limited supply of antibiotics in the hospital, some of the inpatients were asked to buy them from private pharmacies. The amount of antibiotics bought from outside the hospital could not be reflected in this study. For the outpatients, the majority did not receive antibiotics from the hospital pharmacy. Again, data on antimicrobials used by the patients were omitted in the analysis because many patients did not know what medications were receiving and there were no proper records of antimicrobials given in patient files and also in treatment charts.

7.0 Conclusion

Findings of this study show resistance to be high to some of commonly used antimicrobial agents. The trend also shows that there has been a change in the resistance to some antibiotics, erythromycin resistance among *S.aureus* has risen from 2% in 1979 to 34% in the present study, whereas the resistance to other antimicrobials such as penicillin has been persistently high for some years (>80%). A fall in resistance was noted in those antimicrobials that are no longer used in large quantities like tetracycline, whose resistance among *S.aureus* declined from 44% in 1978 to 29.8% in the present study. Notably, resistance to methicillin was 2.1% among *S. aureus*. There was also no vancomycin resistance in both *S. aureus* and enterococci. This is an encouraging finding as MRSA and VRE are causes of substantial morbidity and mortality in developed countries.

Therefore there is a need to limit irrational and indiscriminate use of antibiotics in Tanzania in order to limit the spread of resistance.

S. aureus was found to be the most prevalent organism in most of pyogenic infections. Similar findings have been reported worldwide.

The sample size was not large to draw a conclusion about the difference in the pattern of organisms and their antimicrobial susceptibility patterns in isolates from inpatients and those from outpatients. Hence a larger study is required to ascertain this finding.

MEDICAL LIBRARY DAR ES SALAAM

There was a positive association between consumption of ampicillin and chloramphenicol and the degree of antimicrobial resistance.

8.0 Recommendations

1. There is a need to have a permanent surveillance system established at the MNH so as to know the most prevalent organisms and their sensitivity patterns.
2. Ampicillin should no longer be used for gram positive and negative rods as it has shown to have high resistance. Similarly, penicillin should be replaced in the treatment of staphylococcal infections.
3. Doxycycline, erythromycin, trimethoprim/sulphamethaxazole and amoxycillin/clavulanic acid have shown high resistance among some organisms. They should be used with care and on basis of sensitivity results.
4. Strict prescribing and antibiotic purchasing procedures be enforced to curb the current tide of resistance.
5. Strict regulation should also be put in place to curb importation of substandard medications.
6. The public needs to be educated about antibiotic use and importance of compliance.

9.0 References

1. MacSween RM and Whaley K. Microbial infection. In: *Muir's Textbook of Pathology*, 13th ed, ELBS, 1992: pp293-300.

2. Huskins WC and Soule B. Infection control in countries with limited resources. *Curr Opin Infect Dis* 1998; **11:** 449-455.

3. Orrett FA, Brooks PJ and Richardson EG. Nosocomial infection in a rural regional hospital in a developing country: Infection rates by sites, service, cost and infection control practices. *Infect Control Hosp Epidemiol* 1998; **19:** 136-140.

4. Malonza IM, Omari MA, Bwayo JJ et al. Community acquired bacterial infections and their antimicrobial susceptibility in Nairobi, Kenya. *East Afr Med J* 1997; **74 (3):** 166-70.

5. Asseffa A and Yohannes G. Antibiotic sensitivity pattern of prevalent bacterial pathogens in Gondar, Ethiopia. *East Afr Med J* 1996; **73 (1):** 67-71.

6. Haley RW, Culver DH, White JW et al: The nationwide nosocomial infections rate: A new need for vital statistics. *Am J Epidemiol* 1985; **121(2):** 159-67.

7. Wenzel RP: The mortality of hospital acquired blood stream infections: Needs for a new vital statistics? *Int J Epidemiol* 1988; **17(1):** 225–27.

8. World Health Organization. Resistance to antimicrobial agents. *Weekly Epidemiological Record* 1997; **72(45):** 333-336.

9. Prabhakar P, Rao AB and Hira J. Bacteriological study of diabetic foot ulcers. *Trop Geogr Med* 1981; **33(3):** 249-52.

10. Brook I and Frazier EH. Aerobic and anaerobic bacteriology of wounds and cutaneous abscesses. *Arch Surg* 1990; **125:** 1445-1451.

11. Eltahawy ATA, Mokhtar AA, Khalaf RMF et al. Postoperative wound infection at a university hospital in Jeddah, Saudi Arabia. *J Hosp Infect* 1992; **21:** 79-83.

12. Saha SC, Zaman MA, Khan MR et al. Common aerobic bacteria in post operative wound infection and their sensitivity pattern. *Bangla Med Res Counc Bull* 1995; **21(1):** 32-37.

13. Abussaud MJ and Meqdem MM. A study of some factors associated with wound infection. *J Hosp Infect* 1986; **8(3):** 300-304.

14. Jarvis WR and Martone WJ. Predominant pathogens in hospital infections. *J Antimicrob Chemother* 1992; **29(supp A):** 19-24.

15. Oni AA, Bakare RA, Okesola AO et al. Pattern of bacterial pathogens in surgical wound infections. *Afr J Med med Sci* 1997; **26:** 139-140.

16. Kotisso B and Aseffa A. Surgical wound infection in a teaching hospital in Ethiopia. *East Afr Med J* 1998; **75(7):** 402-405.

17. Shija JK. The incidence and patterns of sepsis among general surgical inpatients at Muhimbili Hospital, Dar es Salaam, 1973. A preliminary report. *East Afr Med J* 1973; **3:** 254-259.

18. Collee JG, Fraser AG, Marmion BP et al (eds). Laboratory strategy in the diagnosis of infective syndrome. In: *Mackie and McCartney Practical Medical Microbiology*, 14th ed. New York, Churchill Livingstone, 1996: pp60-1.

19. Takenawa M, Morikawa Y, Nakagawa T et al. Causative organisms of acute otitis media and acute sinusitis in children and their susceptibility of oral beta-lactam antibiotics. *Jpn J Antibiot* 1999; **52(2):** 162-71.

20. Celin SE, Bluestone CD, Stephenson J et al. Bacteriology of acute otitis media in adults. *JAMA* 1991; **266(16):** 2249-52.

21. Gehanno P, N'Guyen L, Derriennic M et al. Pathogens isolated during treatment failures in otitis. *Pediatr Infect Dis J* 1998; **17(10):** 885-90.

22. Hawke M, Wong J and Krajden S. Clinical and microbiological features of otitis externa. *J Otolaryngol* 1984; **13(5):** 289-95.

23. Iroha EO, Kesah CN, Egri-Okwaji MT et al. Bacterial eye infection in neonates, a prospective study in a neonatal unit. *West Afr J Med* 1998; **17(3):** 168-72.

24. Solberg R, Meberg A and Schoyen R. Neonatal conjunctivitis in a nursery and a neonatal unit. *Tidsskr Nor Laegeforen* 1991; **111(10):** 1230-32.

25. Gigliotti F, Williams WT, Hayden FG et al. Etiology of acute conjunctivitis in children. *J Pediatr* 1981; **98(4):** 531-36.

26. Vichyanond P, Brown Q, Jackson D. Acute bacteria conjunctivitis. Bacteriology and clinical implication. *Clin Pediatr (Phila)* 1986; **25(10):** 506-9.

27. Heggie AD. Incidence and etiology of conjunctivitis in Navy recruits. *Mil Med* 1990; **155(1):** 1-3.

28. Brook I, Pettit TH, Martin WJ et al. Anaerobic and aerobic bacteriology of acute conjunctivitis. *Ann Ophthalmol* 1979; **11(3):** 389-93.

29. Perkin RE, Kundsin RB, Pratt MV et al. Bacteriology of normal and infected conjunctiva. *J Clin Microbiol* 1975; **1(2):** 147-49.

30. Husain MT, Karim QN and Tajuri S. Analysis of infection in a burn ward. *Burns* 1989; **15(5):** 299-302.

31. Atoyebi AO, Sowemimo GO, Odugbemi T. Bacteria flora of burn wounds in Lagos, Nigeria: a prospective study. *Burns* 1992; **18(6):** 448-51.

32. Kalayi GD. Burn injury in Zaria: one year prospective study. *East Afr Med J* 1994; **71(5):** 317-22.

33. Bowen-Jones JR, Coovadia YM and Bowens-Jones EJ. Infection in a Third World burn facility. *Burns* 1990; **16(6):** 445-48.

34. Mousa HAL. Aerobic, anaerobic and fungal burn wound infections. *J Hosp Infect* 1997; **37(4):** 317-23.

35. Bowser-Wallace BH, Graves DB, Caldwell FT. An epidemiological profile and trend analysis of wound flora in burned children: 7 years' experience. *Burns Inc Therm Inj* 1984; **11(1):** 16-25.

36. Vrankova J, Bendova E, Konigova R et al. Bacteriological monitoring in the Prague Burns Center. *Acta Chir Plast* 1998; **40(4):** 105-108.

37. Bowler P. The anaerobic and aerobic microbiology of wounds: a review. *Wounds* 1998; **10(6):** 170-78.

38. Rosenblatt J. Can we afford to do anaerobic culture and ID? Positive point of view. Proceeding of Anaerobe Society of the

Americas Congress on anaerobic bacteria and anaerobic infections, Chicago, 1996.

39. Maselle SY, Mhalu FS, Ipuge YA et al. Surveillance of antibiotic sensitivity of gram positive cocci in a national consultant hospital in Dar es Salaam, Tanzania - a guide to antimicrobial therapy. *Medicom* 1985; **7(1):** 11-13.

40. Ellison MJ. Horner RD, Lucey MJ et al. Outpatient antimicrobial susceptibility: Comparison with inpatient susceptibility patterns. *J Am Bd Fam Pract* 1989; **2 (4):** 223 – 226.

41. Fleming A: On the antibacterial action of cultures of a penicillin, with special reference to their use in the Isolation of B. influenza. *Brit J Exp Path* 1929; **10:**226.

42. Chain E, Florey HW et al. Penicillin as a chemotherapeutic agent. *Lancet* 1940; **2:** 226.

43. Brooks GF, Butel JS and Morse SA (eds). Antimicrobial chemotherapy. In: *Jawetz, Melnick, Adelberg's Medical*

microbiology, 21st ed. Connecticut, Appleton & Lange, 1998: pp.145-175.

44. Tenover FC. The best of times, the worst of times. The global challenge of antimicrobial resistance. *Pharm World Sci* 1995; **17(5):** 149-51.

45. O'Brien TF. The global epidemic nature of antimicrobial resistance and the need to manage it locally. *Clin Infect Dis* 1997; **24(Supp 1):** S2-8.

46. Kariuki SM and Hart CA. Epidemiological aspects of antimicrobial drug resistance. *East Afr Med J* 1997; **74(3):** 124-28.

47. Shears P. A Review of bacterial resistance to antimicrobial agents in tropical countries. *Ann Trop Paediatr* 1993; **13:** 219-226.

48. Kunin CM. Resistance to antimicrobial drugs - A worldwide calamity. *Ann Intern Med* 1993; **118:** 557-561.

49. Cohen ML. Emerging problems of antimicrobial resistance. *Ann Emerg Med* 1994; **24:** 454-456.

50. Tenover FC, McGowan JE Jr. Reasons for the emergence of antibiotic resistance. *Am J Med Sci* 1996; **311(1):** 9-16.

51. Turnidge J. What can be done about resistance to antibiotics? *BMJ* 1998; **317:** 645-47.

52. Hart CA and Kariuki S. Antimicrobial resistance in developing countries. *BMJ* 1998; **317:** 647-650.

53. Belihu A and Lindtjørn B. Increasing incidence of resistance to antimicrobial in Sidama. *Ethiop Med J* 1999; **37:**181-187.

54. Dowse LJ and Prigent BM. Epidemiology of Beta-Lactamases in Africa: Correlation with resistance to Beta-Lactam antibiotics. *Cli Ther*; **13(2):** 243-53.

55. O'Brien TF et al. Resistance of bacteria to antibacterial agents: Report of task force 2. *Rev Infect Dis* 1987; **9 (Supp 3):** S244-260.

56. Mandell GL, Sande MA. Antimicrobial agents: penicillin, cephalosporins and other beta-lactam antibiotics. In:Gilman AG, Rall TW, Nie AS et al (eds). *Goodman and Gilman's. The pharmacological basis of therapeutics*, eighth edition. New York, NY, Pergamon Press, 1990: pp.1065-1097.

57. Urassa WK, Haule EA, Kagoma C et al. Antimicrobial susceptibility of *Staphylococcus aureus* strains at Muhimbili Medical Centre, Tanzania. *East Afr Med J* 1999; **76(12):** 693-95.

58. Pallares R, Dick R, Wenzel RP et al. Trends in antimicrobial utilization at a tertiary teaching hospital during a 15 year period (1978-92). *Infect Control Hosp Epidemiol* 1993; **14(7):** 376 – 82.

59. Huovinen P, Pulkkinen L, Helin HL et al. Emergence of trimethoprim resistance in relationship to drug consumption in a Finnish hospital from 1971 through 1984. *Antimicrob Agents Chemother* 1986; **29(1):** 73-76.

60. Miller GH, Sabatelli FJ, Hare RS et al. The most frequent aminoglycoside resistance mechanisms-Changes with time and

geographic area: A reflection of aminoglycoside usage patterns? *Clin Infect Dis* 1997; **24(Supp 1):** S46-62.

61. Shihab Eldeen A, Araj GF, Thulesius O et al. A comparison of antibiotic consumption and bacterial resistance patterns in Kuwait and Sweden. *APMIS* 1988; **Supp 3:** 59-62.

62. Cometta A, Calendra T, Bille J et al. *Escherichia coli* resistant to fluoroquinolones in patients with cancer and neutropenia. *N Engl J Med* 1994; **330 (17):** 1240-41

63. Maselle SY, Ipuge YA and Mhalu FS. Antimicrobial susceptibility pattern of important urinary bacterial pathogens in Muhimbili Medical Centre, Dar es Salaam. *Dar Med J* 1980; **8(1):** 35-40.

64. Mhalu FS, Mmari PW and Ijumba J. Rapid emergence of El Tor *vibrio cholera* resistant to antimicrobial agents during the first six months of fourth cholera epidemic in Tanzania. *Lancet* 1979; **1:** 345-47.

65. Okeke IN, Lamikanra A and Edelman R. Socioeconomic and behavioral factors leading to acquired bacterial resistance to

antibiotics in developing countries. *Emerg Infect Dis* 1999; **5(1):** 18-27.

66. WHO. Antimicrobial resistance. Fact sheet no 194, 1998.

67. Indalo AA. Antibiotic sale behaviour in Nairobi: A contributing factor to antimicrobial drug resistance. *East Afr Med J* 1997;**74(3):** 171-3.

68. Kunin CM, Lipton HL, Tupasi T et al. Social, behavioral and practical factors affecting antibiotic use worldwide: Report of Task force 4. *Rev Infect Dis* 1987; **9(Supp 3):** S270-285.

69. Tenover FC. Emerging problems in antimicrobial resistance. *J Intraven Nurs* 1995; **18(6):** 297-300.

70. Cohen ML. Epidemiology of drug resistance. Implications for a post-antimicrobial era. *Science* 1992; **257(21):** 1050-55.

71. McGowan JE Jr, Carlet J. Antimicrobial resistance: A worldwide problem for health care institutions. *Am J Infect Control* 1998; **26:** 541-43.

72. Omari MA, Malonza IM and Bwayo JJ et al. Pattern of bacterial infections and antimicrobial susceptibility at the Kenyatta National Hospital, Nairobi, Kenya. *East Afr Med J* 1997; **74 (3):** 134-137.

73. Barrow GI and Feltham RK (eds). In: *Cowan's and Steel's manual for the identification of medical bacteria.* 3rd ed. Cambridge, Cambridge University Press, 1993.

74. NCCLS. Performance standards for antimicrobial disk susceptibility tests. 6th ed; Approved standards. NCCLS 1997; **17(1):** 1-17.

75. Etest technical manual Edition 2000.

76. SPSS inc. The statistical package for the social sciences, Version 9.0. Chicago, IL: SPSS Inc., 2000.

77. Yalcin AN, Bakir M, Bakici Z et al. Postoperative wound infections. *J Hosp Infect* 1995; **29:** 305-309.

78. Pandit DV, Gore MA, Saileshwar N et al. Laboratory data from the surveillance of a burns ward for the detection of hospital infection. *Burns* 1993; **19(1):** 52-55.

79. Dagan R. Clinical significance of resistant organisms in otitis media. *Pediatr Infect Dis J* 2000; **19(4):** 378-82.

80. Panlilio AL, Culver DH, Gaynes RP et al. Methicillin-resistant *Staphylococcus aureus* in U.S. hospitals, 1975-1991. *Infect Control Hosp Epidemiol* 1992; **13:** 582-586.

81. Riley TV and Rouse IL. Methicillin-resistant *Staphylococcus aureus* in Western Australia, 1983-1992. *J Hosp Infect* 1995; **29:** 177-188.

82. Voss A, Milatovic D, Wallrauch-Schwarz C et al. Methicillin-resistant *Staphylococcus aureus* in Europe. *Eur J Clin Microbiol Infect Dis* 1994; **13(1):** 50-55.

83. Sow AI, Wade A, Faye-Niang MA et al. Methicillin-resistant *Staphylococcus areus* in Dakar. *Medicine Tropicale* 1998; **58(2):** 155-57.

84. Mason PR, Kitai I and Chigonde S. Community acquired staphylococcal skin infections in rural areas of Zimbabwe. *Cent Afr J Med* 1991; **37(2):** 56-60.

85. De Neeling AJ, van Leeuwen WJ, Schouls LM et al. Resistance of Staphylococci in the Netherlands: Surveillance by an electronic network during 1989-1995. *J Antimicrob Chemother* 1998; **41:** 93-101.

86. Geyyid A, Lemeneh Y. The incidence of methicillin resistant *S.aureus* strains in clinical specimens in relation to their beta-lactamase producing and multiple-drug resistance properties in Addis Abeba. *Ethiop Med J* 1991; **29:** 149-161.

87. Orrett FA. Methicillin resistance among Trinidadian isolates of community and hospital strains of *Staphylococcus aureus* and their patterns of resistance to non-β-lactam antibiotics. *Jpn J Infect Dis* 1999; **52:** 238-241.

88. Hiramatsu K. The emergency of *Staphylococcus aureus* with reduced susceptibility to vancomycin in Japan. *Am J Med* 1998; **104(5A):** 7S-10S.

89. Martin R, Wilcox KR. *Staphylococcus aureus* with reduced susceptibility to vancomycin-United States, 1997. *MMWR* 1997; **46(33):** 765-766.

90. Smith TL, Pearson ML, Wilcox KR et al. Emergence of vancomycin resistance in *Staphylococcus aureus*. *N Eng J Med* 1999; **340(7):** 493-501.

91. Sieradzki K, Roberts RB, Haber S et al. The development of vancomycin resistance in a patient with methicillin-resistant *Staphylococcus aureus* infection. *N Eng J Med* 1999; **340(7):** 517-523.

92. Ploy MC, Grelaud C, Martin C et al. First clinical isolate of vancomycin intermediate *Staphylococcus aureus* in French hospital. *Lancet* 1998; **351:** 1212.

93. Rupp ME and Archer GL. Coagulase-negative staphylococci: pathogens associated with medical progress. *Clin Infect Dis* 1994; **19(2):** 231-243.

94. Bitkover CY, Marcusson E and Ransjo U. Spread of coagulase-negative staphylococci during cardiac operations in a modern operating room. *Ann Thorac Surg* 2000; **69(4):** 1110-15.

95. Jarløv JO and Høiby N. Coagulase-negative staphylococci in a major Danish university hospital: Diversity in antibiotic susceptibility between wards. *APMIS* 1998; **106:** 411-416.

96. Gray JW and Pedler SJ. Antibiotic-resistant enterococci. *J Infect Dis* 1992; **21:**1-14.

97. Huycke MM, Sahm DF and Gilmore MS. Multiple-drug resistant enterococci: The nature of the problem and agenda for the future. *Emerg Infect Dis* 1998; **4(2):** 239-49.

98. Wade JJ. *Enteroccus faecium* in hospitals. *Eur J Clin Microbiol Infect Dis* 1997; **16(2):** 113-119.

99. Jenkins SG. Mechanisms of bacterial antibiotic resistance. *New Horiz* 1996; **4(3):** 321-332.

100. Nordmann P. Trends in beta lactam resistance among enterobacteriaceae. *Clin Infect Dis* 1998; **27 (Suppl 1):** S100- 6.

101. Quinn JP. Clinical problems posed by multiresistant nonfermenting gram-negative pathogens. *Clin Infect Dis* 1998; **27 (Suppl 1):** S117-24.

102. Hancock RE. Resistance mechanisms in *Pseudomonas aeruginosa* and other nonfermentative gram-negative bacteria. *Clin Infect Dis* 1998; **27 (Suppl 1):** S93-99.

103. Jarlier V, Fosse T and Phillipon A. Antibiotic susceptibility in aerobic gram-negative bacilli isolated in intensive care units in 39 French teaching hospitals (ICU study). *Intensive Care Med* 1996; **22(10):** 1057-65.

104. Seppala H, Klaukka T, Lehtonen R et al. Erythromycin resistance of group A streptococci from throat samples is related to age. *Pediatr Infect Dis J* 1997; **16(7):** 651-56.

105. Aubry-Damon H and Courvalin P. Bacterial resistance to antimicrobial agents: Selected problems in France, 1996 to 1998. *Emerg Infect Dis* 1999; **5(3):** 315-20.

106. Doern GV, Brueggemann AB, Huynh H et al. Antimicrobial resistance with *Streptococcus pneumoniae* in the United States, 1997-98. *Emerg Infect Dis* 1999; **5(6):** 757-65.

107. Pradier C, Dunais B, Carsenti-Etesse H et al. Pneumococcal resistance patterns in Europe. *Eur J Clin Microbiol Infect Dis* 1997; **16(9):** 644-47.

108. Monsen T, Ronnmark M, Olofsson C et al. Antibiotic susceptibility of Staphylococci isolated in blood cultures in relation to antibiotic consumption in Hospital wards. *Scand J Infect Dis* 1999; **31:** 399-404.

109. Gaynes R. The impact of antimicrobial use on the emergence of antimicrobial-resistant bacteria in the hospitals. *Infect Dis Clin North Am* 1997; **11(4):** 757-765.