

ACKNOWLEDGEMENTS

First, I would like to thank God, for making it possible to complete this study.

I have the honor and the pleasure to thank my supervisor Prof. Hassan Abdel Aziz Musa, Dean Faculty of Medical Laboratory Science National Ribat University, who offered me help in all aspects that of this study.

My thanks to all personnel in the Bacteriology Department, Ribat University Hospital, Burri, especially the lab technician Gehad Ibrahim. Also my thanks extended to the lab assistant Habib Mohamed and staff member of the Central Veterinary Research lab. Center, Soba. Who helped me in this study.

I sincerely thank my brothers, other member of the family and colleague for their continuous help.

Moreover, I thank the College of Graduate Studies, Sudan University of Science and Technology, for financial support, and my special thanks to Dr. Humodi A.Saeed the coordinator of the Master Program in Clinical Microbiology.

Last but not least my thanks are extended to the Mohamed Gasm for typing this work.

ABSTRACT

This study was conducted with specific objectives of isolation and identification of bacteria associated with Anesthetic apparatus used during operations or intubations in intensive care units.

Two hundred samples were collected from ten different sites of Anesthetic apparatus, Twenty specimens were taken from each sites were considered sterile and ready to use. The duration of samples collection was between (March 2001 – October 2002). Twenty specimens were taken from each site. The samples were collected from the major emergency complex theatre at Ribat University Hospital and the major theatre of Sahiroon Hospital in Khartoum state.

Bacteriological examinations were performed in the microbiology laboratory, Department of Microbiology, Ribat University Hospital and Central Vet. Research Lab. Center, Soba.

Both Gram-positive and Gram-negative bacteria were isolated, identified and differentiated using standard different biochemical reactions.

The Gram-positive isolates were *Staphylococcus spp.*, *Micrococcus spp.* and *Bacillus spp.* The Gram negative isolate were only *Pseudomonas*.

The commonest bacteria that contaminate the Anesthetic apparatus were *Pseudomonas spp.* (60.37%), *Micrococcus spp.* (20.75%) and *Bacillus spp.* (18.38%).

The antibiotic Sensitivity tests were done for each isolated strain.

Pseudomonas spp. were (100%) sensitive to Amickacin, Pefloxacin and Ciprofloxacin and (72.7%) sensitive to Ofloxacin and (100%) resistant to Ampicillin, Cefoperazone, Piperacillin, Ceftazidime Ceftizoxime, Augmentin, and (72.7%) resistant to Cefotaxime and Gentamicin .

Micrococcus spp. were (85.7%) sensitive to Ciprofloxacin and (71.4%) sensitive to Gentamicin, Ofloxacin and Pefloxacin, intermediately sensitive to Ceftriazone. They were found to be (85.7%) resistant to Cephalexin, (71.4%) to Ampicillin, and Lincomycin and (57.1%) resistant to Roxythromycin and Cefotaxime.

Bacillus spp. were (100%) sensitive to Gentamicin and Ciprofloxacin, (85.7%) to Roxythromycin, (71.4%) to Ofloxacin and Pefloxacin and intermediately sensitive to Cephalexin and Ceftriazone. They were on the other hand found to be (85.7%) resistant to Ampicillin and (57.1%) to Cefotaxime and Lincomycin.

ملخص الأطروحة

اجريت هذه الدراسة بهدف عزل وتعريف البكتريا المصاحبة لتلوث معدات وأجهزة التخدير المستعملة في العمليات الجراحية وحدات العناية المكثفة

تم جمع مائتي عينة من عشرة اجهزة مختلفة من معدات و اجهزة التخدير المعقمة المعدة للاستعمال. بمعدل عشرون عينة لكل جهاز من اجهزة التخدير المختلفة جمعت العينات من الفترة من (مارس 2001م - اكتوبر 2002م). من مجمع العمليات الجراحية والعناية المكثفة بمستشفى الرباط الجامعى و ساهرون التخصصى بولاية الخرطوم.

أجريت كل الاختبارات البكتيرية بالمواصفات الفنية المثلي في معمل الأحياء الدقيقة بمستشفى الرباط الجامعى ومركز معامل الأبحاث البيطرية المركزية -سوبا.

عزلت وعرفت جميع البكتريا الموجبة لصبغة غرام والسالبة لصبغة غرام باستخدام التفاعلات الكيميائية الحيوية المثلي.

البكتريا الموجبة لصبغة غرام والتي عزلت تحتوي على المكورات العنقودية والمكورات السبحية والمايكروكوكس والباسليس.

البكتريا السالبة لصبغة غرام والتي عزلت تحتوي على نوع سودوموناس فقط (الزائفة الزنجارية).

أثبتت الدراسة أن معظم البكتريا التي تصاحب تلوث أجهزة التخدير و اجهزة التنفس الصناعاتى بالعناية المكثفة من نوع سودوموناس (*Pseudomonas*) بنسبة 60.37% ويلبها المايكروكوكس (*Cocci*) بنسبة 75.20% ثم الباسليس (*Bacillus*) بنسبة 38.18%.

ثم أجريت لكل عينة اختبارات حساسية المضادات الحيوية والتي أثبتت الدراسة بان سودوموناس (*Pseudomonas*) المعزولة حساسة لمضاد اميكاسين (Amikacin) و سيبروفلوكساسين (Ciprofloxacin) و بيفلوكساسين (Pefloxacin) بنسبة (100%) و حساس لمضاد افولكساسين (Ofloxacin) بنسبة (72.7%) و مقاوم لمضاد امبسيلين (Ampicillin) و سيفوبرازون (Cefoperazone) وبييراسيلين (Piperacillin) و (Ceftazidime) وسفتازدايم وسيفوتاكسيم (Ceftizoxime) و او قمنتين (Augmentin) بنسبة (100%) ومضاد جنتاميسين (Gentamicin) وسفتاكسايم (Cefotaxime) بنسبة (72.7%).

اما المايكروكوكس (*Micro Cocci*) حساسة لمضاد سيبروفلوكساسين (Ciprofloxacin) بنسبة (85.7%) و افولكسين (Ofloxacin) و بيفولكسين (Pefloxacin) وجنتاميسين (Gentamicin) بنسبة (71.4%) وسفتازون (Ceftriazone) بنسبة (57.1%) ومقاومة لمضاد سفالكسين (Cephalexin) بنسبة (85.7%) ولمضاد امبسيلين (Ampicillin) ولانكوماسين (Lincomycin) بنسبة (71.4%) و لمضاد اروثرومايسين (Roxythromycin) و سفتاكسايم (Cefotaxime) بنسبة (57.1%).

اما الباسيليس (*Bacillus*) حساسة لمضاد سيبروفلوكساسين (Ciprofloxacin) و جنتاماسين (Gentamicin) بنسبة (100%) و اريثرومايسين (Roxythromycin) بنسبة (85.7%) وتمضاد افولكسين (Ofloxacin) و بيفلوكسين (Pefloxacin) بنسبة (71.4%) و لمضاد سفالكشين (Cephalexin) وسفتازون (Ceftriazone) بنسبة (57.1%). ومقاومة لمضاد امبسيلين (Ampicillin) بنسبة (85.7%) ومضاد لانكوماسين (Lincomycin) وسفتاكسايم (Cefotaxime) بنسبة (57.1%).

LIST OF CONTENTS

	Page
Acknowledgement	i
Abstract English	ii
ملخص الأطروحة	iv
Table of Contents	vi
List of Tables	xi
List of Figures	xii
List of Photos	xiii
CHAPTER ONE	
1. Introduction and Literature Review	1
1.1 Nosocomial infections	1
1.2 Bacteria associated with anesthetic equipments	2
1.2.1 Gram- positive bacteria	2
1.2.1.1 <i>Staphylococcus spp</i>	2
1.2.1.1.1 Anti-microbial sensitivity	3
1.2.1.2. <i>Streptococcus spp.</i>	4
1.2.1.2.1 <i>Streptococcus pyogenes</i>	4
1.2.1.2.2 <i>Streptococcus pneumoniae</i>	4
1.2.1.2.2.1 Anti-microbial sensitivity	5
1.2.1.3 <i>Bacillus spp</i>	5
1.2.1.3.1 Anti-microbial sensitivity	5
1.2.2 Gram- negative bacteria	5
1.2.2.1 <i>Pseudomonas spp</i>	5
1.2.2.1.1 <i>Ps. aeruginosa</i>	6
1.2.2.1.2 <i>Ps. maltophilia</i>	7
1.2.2.1.3 <i>Ps. cepacia</i>	7
1.2.2.1.4 <i>Ps. mallei</i>	7
1.2.2.1.5 <i>Ps. pseudomallei</i>	7
1.2.2.2 Anti-microbial sensitivity	7
1.3 Anesthetic equipments	8

1.3.1	Anesthesia systems	8
1.3.2	Breathing circuit	8
1.3.3	Patient circuit	8
1.3.4.	Anesthesia equipments types and uses	8
1.3.4.1	Anesthesia machine	8
1.3.4.2	Ventilators	9
1.3.4.3	Scavenging system	9
1.3.4.4	Laryngoscopes	9
1.3.4.5	Endo tracheal tubes	10
1.3.4.6	Air ways	10
1.3.4.7	Face mask	10
1.3.4.8	Laryngeal mask	11
1.3.4.9	Laryngeal spray	11
1.3.4.10	Intubating forceps	11
1.3.4.11	Cuff tubes	11
1.3.5	Infection control procedures for Anesthesia equipments	11
1.4.5.1	Effective strategies for infection control programs	12
1.3.5.2	Risk of transmitting infection	13
1.3.5.3	Steps in decontamination plan	14
1.3.6	Prevention of nosocomial infection in patients	14
1.3.6.1	Cleaning	14
1.3.6.2	Antiseptics	15
1.3.6.3	Decontamination	15
1.3.6.4	Disinfection	16
1.3.6.4.1	High-level disinfection	16
1.3.6.4.2	Intermediate-level disinfection	17
1.3.6.4.3	Low -Level disinfection	18
1.3.6.5	Sterilization	18
1.4	Justification & Objectives	20
1.4.1	Justification	20
1.4.2	Objectives	20
1.4.2.1	General	20

1.4.2.2	Specific	20
	CHAPTER TWO	
2.	Materials and Methods	21
2.1	Materials	21
2.1.1	Anesthetic equipments	21
2.1.2	Culture equipments	21
2.1.3	Other Materials	22
2.2.	Media for isolation & identification & sensitivity testing	22
2.2.1	Solid media	22
2.2.1.1	Nutrient agar	22
2.2.1.2	Blood agar	22
2.2.1.3	MacConkey agar	22
2.2.1.4	Urea agar base	23
2.2. 1.5	Simmon citrate	23
2.2..1.6	Diagnostic sensitivity test (DAT) agar	23
2.2. 1.7	Gelatin media	24
2.2.2	Semi -solid media	24
2.2.2.1	Motility medium	24
2.2.3	Liquid media	24
2.2.3.1	Nutrient broth	24
2.2.3.2	Peptone water	24
2.2.3.3	Carbohydrate fermentation	25
2.3	Reagents	25
2.3.1	Hydrogen peroxide	25
2.3.2	Tetra methyl -p- phenylenediamine dihydrochloride	25
2.3.3	Kovacs reagent	25
2.4	Blood	26
2.5	Methods	26
2.6	Collection of samples	26
2.6.1	Distribution of samples	27
2.7	Culture methods	27
2.7.1	Primary inoculation on solid media	27

2.7.2	Subculture of primary isolates	27
2.7.3	Incubation of culture	27
2.7.4	Examination of the cultures	27
2.8	Identification of the isolated bacteria	28
2.8.1	Primary culture	28
2.8.1.1	Preparation of smears	28
2.8.1.2	Gram-staining	28
2.8.2	Secondary identification of isolated bacteria	28
2.8.2.1	Oxidase test	28
2.8.2.2	Catalase test	28
2.8.2.3	Motility test	29
2.8.2.4	Sugar fermentation test	29
2.8.2.5	Crease test	29
2.8.2.6	Peptone water	29
2.8.2.7	Tube coagulase test	29
2.9	Antibiotic sensitivity test	30
	CHAPTER RHREE	
3.	Results	33
3.1	Types of bacteria isolated from anesthetic equipments	33
3.1.1	The species of isolated <i>Pseudomonas</i>	33
3.1.2	<i>Bacillus</i> spp.	33
3.1.3	Gram-positive <i>Cocci</i> spp.	33
3.2	Biochemical properties of the bacteria isolated from the anesthetic equipments	34
3.3	Invitro antimicrobial sensitivity	34
	CHAPTER FOUR	
4.	Discussion	52
	CHAPTER FIVE	
5.	Conclusion & Recommendation	55
5.1	Conclusion	55
5.2	Recommendations	56
	References	57

LIST OF TABLES

		Page
Table 1	Multi disk for antimicrobial susceptibility testing for Gram Negative bacteria	31
Table 2	Multi disk for antimicrobial susceptibility testing for Gram Positive bacteria	32
Table 3	<i>Pseudomonas spp</i> isolated from the anesthetic equipment	36
Table 4	<i>Bacillus spp</i> isolated from the anesthetic equipments	36
Table 5	Gram-positive <i>cocci spp</i> isolated from the anesthetic equipment	36
Table 6	Biochemical reaction & character for isolation & identification of gram –positive bacteria	37
Table 7	Biochemical reaction & character for isolation & identification of gram-negative bacteria	38
Table 8	Antimicrobial Susceptibility Testing for Gram - Positive Bacteria	39
Table 9	Percentage of Antimicrobial Resistance of the different gram - positive isolated	40
Table 10	Antimicrobial Susceptibility Testing for the Gram – Negative Bacteria isolates	41
Table 11	Percentage of Antimicrobial Resistances of the different Gram – Negative isolates	42

LIST OF FIGURES

		Page
Fig. 1	Total number of isolates from the different items of anesthetic equipments.	43
Fig. 2	The Percentage of growth from the anesthetic equipments.	44
Fig. 3	Types of bacteria isolated from the anesthetic equipments.	45
Fig. 4	Percentage of the different types isolated from the anesthetic equipments.	46
Fig. 5	<i>Pseudomonas spp.</i> isolated from the anesthetic equipments.	47
Fig. 6	<i>Bacillus spp.</i> isolated from the anesthetic equipments.	48
Fig. 7	Gram positive <i>Cocci</i> isolated from the anesthetic equipments	49
Fig. 8	Percentage of antimicrobial resistance of the different of Gram positive isolates.	50
Fig. 9	Percentage of the antimicrobial resistance of the different species <i>Pseudomonas</i> .	51

APPENDIX 1 - LIST OF PHOTOS

		Page
Photo. 1	Facemask	59
Photo. 2	Air ways	60
Photo. 3	Laryngoscope blade	61
Photo. 4	Suction catheters (or Tube).	62
Photo. 5	Tracheal tubes.	63
Photo. 6	Magill intubating forceps.	64
Photo. 7	Connection machine line.	65
Photo. 8	Anesthetic machine lines.	66
Photo. 9	Embo-Bags.	67
Photo. 10	Tracheal tube cuff.	68
Photo. 11	<i>Staph. epidermidis</i> isolated from anesthetic equipments.	69
Photo. 12	<i>Pseudomonas spp.</i> isolated from anesthetic equipments.	70
Photo. 13	<i>Bacillus spp.</i> isolated from anesthetic equipments.	71
Photo. 14	<i>Strepto. spp.</i> isolated from anesthetic equipments.	72
Photo. 15	Sugar test for biochemical reaction.	73