CONJUNCTIVAL NORMAL FLORA AMONG KENYANS

A dissertation submitted as part fulfilment
for the degree of Masters of Medicine (Ophthalmology),
University of Nairobi

By

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2005

DECLARATION

This dissertation is my original work, and has not been presented for a degree at any other university.

Signed: 4-JEzll - Date: , 07 , 66

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(Candidate)

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APPROVAL

This thesis proposal has been submitted to the ethical committee with an approval as University Supervisors.

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DEDICATION

This book is dedicated to my parents, Shabbir and Fatema Jafferji who have nurtured me, disciplined me and taught me to set my goals and standards high, who have been there through the thick and thin of my life, to my loving and supporting wife Tasneem Jafferji

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LIST OF ABBREVIATIONS

AJO	American Journal Ophthalmology
P	Beta
BA	Blood Agar
Braz	Brazil
BHI	Brain Heart Infusion
CA	Chocolate Agar
CALT	Conjunctival associated lymphoid tissue
CNS	Coagulase Negative Staphylococcus
g	Grams
ICU	Intensive Care Unit
IgA	Immunoglobulin A
IgG	Immunoglobulin G
J	Journal
KOH	Potassium Hydroxide
Microbiol	Microbiology
mis	milliliters
Sp	Species
Thio	Thioglycolate

SUMMARY

Background: The most dreaded complication of intraocular surgery is endophthalmitis an ophthalmic emergency and the commonest organisms responsible for this infection are Staphylococcus sp. and Streptococcus sp. Both these organisms have been shown to be part of the normal conjunctival flora which may invade the globe such as during surgery (evidence based from genetic studies of the organisms isolated). Moreover there are regional differences and shift in the antibiotic sensitivity pattern with time. A study demonstrating the normal flora in our region and the associated antibiotic sensitivity pattern provides important information for the prevention and early management.

Objectives: The study aims to determine the pattern of normal flora inhabiting the conjunctiva, possible changes with time in the pattern of the flora and determine the changing antibiotic sensitivity pattern.

Methodology: In a descriptive retrospective study, all data on ocular normal flora obtained by the Department of Ophthalmology microbiology laboratory between 1994 and 1997 was reviewed while observing the strictest patient confidentiality and observing the laboratory and study protocols. Data was analyzed in descriptive form using SPSS 11.5 statistical analytical programme and Microsoft excel where applicable

Results: out of 264 questionnaires reviewed 43.94% samples were positive. The age range was from 11-95 years and the commonest organism isolated in our region was CNS followed by S.aureus. There were a total of 13 species isolated. When comparing the floral distribution as per the year of collection, there was no statistically significant difference in the pattern of flora from one year to another. Antibiotic sensitivity patterns for CNS and S.aureus were done. The trends showed an increase in the sensitivity of the commonly used antibiotics in the region during the study period.

UST OF TABLES & FIGURES

1. INTRODUCTION AND LITERATURE REVIEW

Bacterial infections are an important cause of ocular morbidity. A number of factors are involved in the complex pathogenesis of ocular infections.

Up to the time of birth, the healthy human fetus has no microbial growth on the ocular surface. It acquires the microbes from the mother's birth canal or from the immediate surrounding environment after birth. These organisms then multiply in the favourable atmosphere and form the normal flora on the body surfaces like the conjunctiva. Although harmless, they can cause disease patterns once the defense patterns of the ocular surfaces are compromised. Factors that enhance the organisms to thrive maybe different in different populations, and an organism which may cause mild symptoms in one individual may cause a serious infection in another [32,33].

The micro flora of the outer segments (eyelids, margins of the eyelids with the cilia, conjunctiva, cornea and ductus lacrimalis) correspond largely to the flora of the skin[2,18] .It is thought that the cilia and the excretory glandular ducts of the lid edge are responsible for the germ release which may colonise the conjunctiva subsequently[2,19].

In a study conducted in Argentina on the ocular flora of the newborn by Eder M and Mino de Kasper, on 187 patients, where samples were taken 48hrs after birth, 176 samples were positive in the vaginal delivery group whereas 65 patients in the caesarean section group. Coagulase negative Staph. Aureus (CNS) was the predominant species with 38% in vaginal delivery group and 68% in the caesarean section group. Other major species included Propionibacterium sp (19% and 33% respectively), Corynebacterium sp (7% and *9%* respectively). Altogether 16 species were isolated [39]

The two main differences between ocular microbiology and general clinical microbiology are:

1 Many of the organisms responsible for ocular infections are either part of normal flora or occasionally contaminants.

2. The small size or quantity of ocular specimen make recovery and hence identification of the organisms difficult. As many species do not withstand transport they require immediate inoculation in suitable media for further microbiological processing and preferably at specialized facilities.[1]

1.1 CONJUNCTIVA

The conjunctiva is a vascularized membrane covering the anterior surface of the globe, which consists of epithelial and stromal layers. The epithelial layer is continuous with the epidermis of the lids at the lid margin and the epithelium of the cornea at the limbus.[31]

The conjunctiva has enormous potential for combating infection due to the fact that:

- It is highly vascularized
- Different types of cells initiate and participate in defense inflammatory reactions
- It contains immunocompetent cells which contribute a rich supply of immunoglobulins
- The microvilli and enzymatic activity which enable it to engulf and neutralize organisms including viruses[31]

1.1.1 BASIC ANATOMY

The conjunctiva lines the posterior surface of the lids and the anterior surface of the globe at the same time forming 2 recesses, the superior fornix approximately 8-10mm from the limbus and the inferior fornix some 8-9mm.

Medially the fomicial structures are replaced by the caruncle and the plica semilunaris. Laterally the fornix is quite deep extending beyond the equator of the globe approximately 14mm from the limbus.

Clinically the conjunctiva is divided into the palpebral, bulbar and the fornicial conjunctiva.

1.1.2 CONJUNCTIVAL EPITHELIUM

Stratified squamous epithelium rests on a very loose connective tissue, the substantia propria. The surface epithelial cells contain numerous microvilli

The tarsal conjunctival epithelium is about 5-6 layers thick. The basal epithelium of the fornices tends to be more columnar than the palpebral which is more cuboidal.

The limbal conjunctival epithelium is about 10-15 cell layers thick. Goblet cells are absent here and the epithelium tends to become more like the corneal epithelium. It shows numerous lymphocytes and Langerhans cells.

1.2 NORMAL OCULAR FLORA

Normal ocular flora constitutes those organisms which are present on the eyelid and conjunctiva without causing any diseases. These organisms are considered to be saprophytic without causing any diseases but have the potential to become pathogenic when the normal defense mechanisms are faultered.[2, 7] The normal conjunctival flora appears to be derived from the skin and it establishes itself a few weeks after birth forming an equilibrium within the sac. This is protective against the proliferation of pathogenic bacteria. [34]

In conjunctival smears, the bacterial species widely predominate over fungal, parasitic or viral elements. In fact viruses if found are considered to be pathogenic as they are not found to be part of the normal ocular flora.[2] Forty six to Seventy five percent of the cases contain potentially pathogenic strains of bacteria. Out of the over Sixty five validated types of the bacteria of the conjunctiva, gram positive bacteria predominate. These include the CNS genuses, Propionibacterium and Corynebacterium species as well as Peptostreptococcus, Streptococcus and Actinomyces families. Gram negatives include Neisseria, Haemophillus and Proteus sp. from the Enterobacteriaceae family [5]

A study carried out by Ta, Chang and Singh, examined the antibiotic susceptibility patterns of conjunctival bacterial flora isolated preoperatively from patients undergoing anterior segment surgery. Out of the One hundred and twenty eyes studied, Eighteen percent showed no bacterial

growth. One hundred and forty three bacterial strains were isolated from the remaining eyes, of which 78% were coagulase-negative Staphylococci (CNS). Among the CNS, greater than Ninety percent were susceptible to cefotaxime, levofloxacin, imipenem, meropenem, vancomycin and each of the aminoglycosides except neomycin. Between Seventy and Ninety percent of the CNS were susceptible to cefazolin, neomycin, ciprofloxacin, ofloxacin, norfloxacin and chloramphenicol. Less than Seventy percent of the isolated CNS were sensitive to the penicillin analogues, ceftazidime, erythromycin and tetracycline. The authors concluded that of the tested antibiotics, preoperative conjunctival isolates of CNS seem to be most sensitive to vancomycin, the aminoglycosides (except neomycin) and levofloxacin.[20]

In a recent study done on the antibiotic susceptibility pattern of bacterial ocular flora by Mino de Kasper et al on One hundred and sixty four patients prior to undergoing surgery, they isolated bacteria in One hundred and sixty two patients. The commonest bacteria isolated was CNS (76%) with Two percent resistant to Gatifloxacin and Moxifloxacin whereas none were susceptible to minocycline or vancomycin. Other species isolated included nineteen S.aureus, eleven Gram negative rods, eight Streptococcus Group D. most Staph, aureus were susceptible to all antibiotics accept penicillin and macrolides. The Strep. Group D were sensitive to Flouroquinolones, Mezlocillin, Imipenem, and Vancomycin. All the gram negative rods were susceptible to flouroquinolones. Approximately half of all the bacteria were resistant to Erythromycin, and one in every 3 patients harboured a strain of multi-resistant strain of bacteria. It was concluded from this study that Flouroquinolones provide an excellent cover against conjunctival bacterial flora.[40]

Trindade et al, who studied the conjunctival normal flora of hospital workers, commented that the conjunctiva has a peculiar resident micro biota predominantly consisting of Diphtheroids (Corynebacterium xerosis), Neisseria, Haemophilus-like Gram-negative bacilli (Morax-Axenfeld bacillus, a Moraxella species), Staphylococci and non-hemolytic Streptococci. The normal microbiota also included Staphylococcus aureus, Propionibacterium sp, Peptococcus sp, Peptostreptococcus sp, Clostridium sp, Fusarium sp, and Cephalosporium sp.[23, 24, 25, 26, 27, 28, 29] This showed a predominantly Gram negative pattern of normal flora in hospital workers unlike that seen in the normal population

In a study conducted by Martins Alveranga et al on the aerobic conjunctival flora of diabetic patients. A diabetic patient cohort was compared with non diabetic subjects. The frequency of positive conjunctival cultures was significantly higher in the diabetic group (94.18%) than in the nondiabetic group (73.33%). Among diabetic patients, a significantly higher frequency of positive cultures was detected in those with diabetic retinopathy than in those without retinopathy. Neither the duration of the diabetes nor the hypoglycemic therapy correlated with the culture results. Coagulase-negative Staphylococcus was the most common microorganism isolated, and its identification was more frequent in patients with retinopathy than in those without diabetic retinopathy. They concluded that the diabetic patients had a significantly higher number of positive conjunctival cultures and the presence of diabetic retinopathy was correlated with an increase in positive cultures and a higher proportion of coagulase-negative Staphylococcus.[30]

The normal bacterial flora of both the eyes is strikingly similar. The eyes are not randomly colonised but are colonised at the same time by the same organisms. The presence of an organism in one eye is strongly suggestive of its presence in the other eye.[1,2]

As the eyelids contribute to the conjunctival sac flora the type of micro organism present in the eyelid and conjunctiva are similar, though they are recovered in greater numbers from the eyelids.

Viruses though not part of the normal flora of the outer segment of the eye, they can occur after viral infections especially of the eye itself. However they can contaminate the conjunctiva when the eye comes into contact with fingers contaminated by other infected lesions. Physiologically the inner segment of the eye is sterile.[2]

Anaerobic bacteria have been reported in normal and infected conjunctiva. Their significance as pathogens is uncertain. Compos in Denmark found AIDS patients to have the highest incidence with different spectrum of anaerobic organisms in a group of AIDS patients with anophthalmia compared to the normal eyes. Anaerobic organisms are normal flora in the normal conjunctival sac and anophthalmic socket.[36,37]

Generally the conjunctival flora is sparse and about Seventeen to Forty nine percent of the cultured samples are usually negative. However this depends largely on the technique applied for sampling and processing of specimen [5,36]

1.3.1 RESIDENT OCULAR FLORA

These are permanent flora which represents true colonisation and repeated cultures usually reveal the same organism in large numbers. Resident ocular flora typically includes:

1. Staphylococcus epidermidis
2. Diphtheroids
3. Staphylococcus aureus(recovered from 33% of normal eyes)
4. Lactobacillus species
5. Propionibacterium species(anaerobic diphtheroid), predominant resident anaerobe[1]

1.3.2 TRANSIENT OCULAR FLORA

These inhabit the eye for short periods and cannot be consistently recovered in consecutive cultures. These transient flora are influenced by factors like:

1. Increased recovery of fungal elements from the conjunctival sac of people living in rural areas
2. Increased recovery of Pseudomonas sp. from hospital patients
3. Increased recovery of Streptococcus, Pneumococcus and Haemophilus in children.

Patients with certain ocular diseases tend to harbour different flora from the normal that is seen in keratoconjunctivitis sicca, the cases have more of Proteus and Staph, aureus. Those on topical and systemic immunosuppressive therapy have an altered pattern of normal flora. Studies have revealed an increase in the the recovery of fungi from the conjunctiva of people on immunosuppressive medications.[1,2]

When changes occur in the environment, commonly occurring microorganisms may temporarily become part of the transitory conjunctival microbiota, without causing damage. However, when a

break occurs in the equilibrium between the resident and transitory microbiota, diseases may arise. These modifications mainly result from the indiscriminate use of eye drops containing antimicrobial agents or corticosteroids. Corticosteroids, in turn, by reducing host resistance may increase the virulence of species known to be pathogenic and may permit other species considered to be commensal to manifest virulence.[21,22]

The transient ocular flora often includes:

1. Staphylococcus aureus
2. Staphylococcus species
3. Bacillus species
4. Haemophilus species
5. Branhamella catarrhalis
6. Enterobacteriaceae: E.coli, Klebsiella species, Enterobacter species.
7. Pseudomonas aeruginosa
8. Anaerobes: Peptococcus, Bacteroides, Clostridium, Actinobacterium, Eubacterium
9. Fungi: Aspergillus, Penicillium, Aternaria, Caldosporium, Candida, Helminthosporium, Rhodotorula, Fusarium species.

Because of its constant exposure to the external medium, the conjunctiva is subject to intense microbial contamination. Most microorganisms are removed by lacrimation, with only a relatively low-density of the microbiota being left behind and a reduced number of species [21,22]

1.4 DEFENSE MECHANISMS

Several defense mechanisms, immunologic and nonimmunologic provide protection against colonization, infection, or invasion of the ocular structures.[6,7]

They can be divided into:

1. anatomic barriers
2. humoral immune response
3. cellular immune response

1.4.1 ANATOMIC BARRIERS

They include the following:

1.4.1.1 EYELIDS

The eyelids are an anatomic barrier that protects the eye against the external environment, during sleep they prevent desiccation of the cornea and conjunctiva.

The blinking reflex protects against air borne organisms, foreign bodies and minor trauma. The lashes sense an approaching foreign body and initiate the blink reflex to protect the eye.

They also help retain the protective tear film to avoid exposure and drying of the ocular surface The wiper effect cleanses the external surface, flushing away mucus with trapped foreign matter in the direction of the inner canthus[8,7]

1.4.1.2 TEAR FILM

The tear film consists of the middle aqueous layer secreted by the lacrimal and the accessory lacrimal glands, the superficial oily layer secreted by the meibomian glands and the underlying mucus layer secreted by goblet cells.

The mechanical flushing action is probably the most important function which prevents adherence of micro organisms to the eye. Mucus traps and acts as a carrier of foreign matter.

The tear film also contains immunoglobulins, lysozymes, P-lysin, lactoferrin and complement. Their presence in the tear film of both normal and pathologic conditions has been well documented, yet the exact role they play in combating diseases are controversial.[6,7,9,10,11,12]

1.4.2 CONJUNCTIVAL SURFACE

Conjunctival epithelium, 10-15 layers thick provides an anatomic barrier to penetration of organisms along with the tear film cover. In addition the conjunctiva has a subepithelial layer of conjunctival associated lymphoid tissue (CALT). The conjunctival epithelium is occasionally infiltrated with lymphoid cells as well[7,13,14]

1.4.3 HUMORAL IMMUNE RESPONSE

1.4.3.1 IMMUNOGLOBULINS

1.4.3.1 (a) IMMUNOGLOBULIN A

IgA is a secretory immunoglobulin which is produced by plasma cells located in the lacrimal gland.

Its main role is in the prevention of adherence of bacteria to the epithelial cells by trapping the organism at the mucus membrane surface and coating it. This is particularly important in the prevention of adherence by Nisseria, Pseudomonas and Chlamydia [7,17]

It does not cause cytolysis or phagocytosis directly but may do this in combination with other tear components. It is also thought to neutralize toxins and is crucial in the neutralization of viruses [12]

1.4.3.1(b) IMMUNOGLOBULIN G

This is the second line of defense against both bacteria and viruses, exerting its action through the classic complement pathway. It forms and fixes complement resulting in the lysis of the pathogen and phagocytosis.[7,15]

1.4.3.2 COMPLEMENT

1.4.3.3 LYSOZYME

Lysozyme is present in relatively high concentration in tears and this has been recognized since 1922, when it was noted by Flemming and Allison.[7,16] Lysozyme comprise Twenty to Forty percent of protein content of tears.[7,12] It is secreted by the apex of the lacrimal acini

It is bacteriolytic against gram positive organisms because of its action against the polysaccharide moiety in the cell wall. Gram negative bacteria are not susceptible due to the presence of lipopolysaccharide over the polysaccharide moiety which acts as a protective covering. However immunoglobulins complement and other tear components may alter the coating to such an extent that the bacteria may be rendered susceptible to the action of lysozyme.

Lysozyme is also a chitinase. It thus breaks down the chitinous cell wall of fungi. This action is enhanced by the chelating action of lactoferrin.

Lysozyme has a limited role in prophylaxis against bacterial infections. Egg white, which is rich in lysozyme, appears to play a theoretical role in the treatment of certain eye infections. In practice, however its role is insignificant and tear lysozyme appears to play a minor role in the prevention of infections of the cornea or the conjunctiva.[7]

1.4.3.4 P-LYSIN

It tends to be active against several of the bacteria that are resistant to the action of lysozyme. Its activity is against cell membrane therefore it leads to cytolysis. It's more highly concentrated in tears than in plasma though platelets are known to be the source of P-lysin.[7]

1.4.3.5 LACTOFERRIN

Lactoferrin can bind reversibly to each of the following: iron, copper, IgA, IgG, and albumin. The virulence of several bacteria especially gram negative tend to increase in the presence of iron. Virulence of Staphylococci, Pseudomonas and gram negatives is altered by the iron binding to lactoferrin and renders them more susceptible to other enzymes such as lysozyme.[7]

1.4.4 CELLULAR IMMUNE RESPONSE

1.4.4.1 LANGERHANS CELLS

They possess the characteristics of macrophages, and they are felt to be important in immunologic surveillance. They are the front line cells in the recognition of and processing of foreign antigens for the presentation to T helper cells with which they interact closely.[7,16]

1.4.4.2 LYMPHOCYTES

T and B lymphocytes are present in the substantia propria of the conjunctiva. They are important in the humoral and cell mediated immune responses. Once a lymphocyte is stimulated by antigen, it forms a clone against that antigen and multiplies and recognizes the antigen much quicker and handles it more efficiently on subsequent exposures.

Subpopulations of T lymphocytes can modify antibody production by B cells. Then an antigen is introduced to the immune system, the lymphocytes become sensitized and retain a memory of the antigen for a long time. Whenever the ocular surface again recognizes such an antigen, it can mount an accelerated immune response ultimately leading to the elimination of the pathogen efficiently.[7,17]

2. STUDY RATIONALE

The course of eye infection is often rapid and the outcome of delayed and inappropriate treatment, especially with infections involving the posterior segment of the eye, can be poor. Very little is known concerning the bacteriology of the conjunctival normal flora in our region. A review of literature shows that most studies have been carried out in other parts of the world, often developed countries and the results on the conjunctival normal flora and their sensitivity patterns may not be applicable to our specific setup, since they are known to vary vastly from region to region.

Knowledge of the normal flora of the conjunctiva and the changing resistance and sensitivity patterns is important as these microbiota can be responsible for the invasion of the ocular structures when defense mechanisms are compromised. This will assist practicing Ophthalmologists in the region in their choice of appropriate antibiotic treatment prior to culture and sensitivity results, and especially so in regions where laboratory facilities are not readily available. It may also assist in reducing the injudicious use of antibiotics. As anti-microbial resistance pattern vary with time and are influenced by prescribing characteristics, susceptibility information of the probable infecting organism is required for proper patient management. Also, the study may have an impact on economic aspect in the management of ocular infections since this may alter prescribing behaviour of the Ophthalmologists in the region.

The study will give a deeper understanding of possible changes of the antibiotic resistance patterns of the conjunctiva in the region.

There is no study done showing the normal conjunctival flora and the changing antibiotic sensitivity patterns for this region.

3. AIM

The aim of the study was to determine the normal bacterial flora inhabiting normal conjunctiva, possible changes in the pattern over time and determine the antibiotic sensitivity patterns.

4. OBJECTIVES

1. To determine the spectrum of normal bacterial flora inhabiting the normal conjunctiva.

2. To determine the changing drug sensitivity patterns for the commonly occurring ocular normal flora in the normal conjunctiva

3. To find out if there are any possible changes in the pattern of ocular flora inhabiting the conjunctiva with time

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5 METHODOLOGY

5.1 TARGET POPULATION

people living in Kenya between January 1994 and December 1997

5.2 STUDY POPULATION

Asymptomatic volunteers at KNH and KEU whose slit lamp examination of the anterior segment had been shown to be normal with no features of ocular infections or ocular surface abnormalities.

5.3 CASE DEFINITION

Any person with a positive culture which was defined as having grown a colony of bacteria within 48hrs of incubation at 37°C on Blood agar, Chocolate agar and/or Thioglycolate agar who had not been on any antibiotic treatment in the preceding one month

5.4 STUDY PERIOD

January 1994 to December 1997

5.5 STUDY AREA

Microbiology laboratory records at the Department of Ophthalmology, Microbiology Laboratory

5.6 STUDY DESIGN

Descriptive retrospective study

5.7 MATERIALS

1. Study Questionnaires
2. SPSS 11.5 statistical analytical programme
3. Personnel: Principal Investigator, Statistician,
4. Equipment: Personal computer, stationery

5.8 STUDY PROTOCOL

The data was collected at the microbiology laboratory in the Department of Ophthalmology, University of Nairobi from volunteers without any signs or symptoms of ocular surface disorders presenting at the microbiology laboratory in the years 1994 to 1997. Other data collected in 1995 was from preoperative patients without ocular surface disorders who were scheduled for intra ocular surgery at the Kikuyu Eye Unit. An informed verbal consent was taken from these people prior to sampling. A detailed history and thorough anterior segment examination was carried out prior to collection of the samples (refer to Appendix II on relevant history and examination findings).

The samples were inoculated immediately on culture plates as per the protocol described below and in appendix I. The samples from KEU were then transported immediately to the microbiology laboratory at the Department of Ophthalmology, where the plates were incubated immediately and slides were stained for microbiological examination. Where appropriate, further cultures or slides were prepared from the sample collected and stored in the container (refer below).

Setup - microbiology laboratory at Department of Ophthalmology, University of Nairobi

Specimen - wet BHI conjunctival swab from the lower fornix

Volume - one swab from each patient

Collection - sterile moist (moistened in BHI broth) cotton wool swab rolled across the lower conjunctival fornix

Storage instructions - directly inoculated on the culture media

Informed verbal consent was obtained from all individuals. A detailed history was taken from the individuals and a thorough ocular surface examination was carried out on the slit lamp to exclude any infections or ocular surface diseases.

Specimen collection and processing:

1. Inoculation of the conjunctival swab for microscopy was carried out as follows: samples from the conjunctiva were collected by rolling the moistened (BHI broth) swab stick in the lower conjunctival fornix from the medial to the lateral canthus and smearing it directly on the naked glass slide to make a thin film. Naked glass slides were then gram stained and examined under a microscope for micro organisms.

2. Inoculation of the conjunctival swab for culture and sensitivity was carried out as follows: inoculation was done after wetting the swab with BHI broth. The swab was then taken from the lower conjunctival fornix. Inoculation in the Petri dish was done in the following order: chocolate agar, followed by blood agar, followed by Thioglycolate agar.

3. Reading of the Petri dish and the broth was done after 24 and 48 hrs of inoculation after which the dishes were discarded.

4. The questionnaires were completed as per the findings on the Petri dishes and the sensitivity patterns were recorded.

BA and CA were used as enriched media whereas Thioglycolate broth was used as differential medium (for both aerobes and anaerobes). Thio broth was used for anaerobic organisms. BHI and Thio broth were used as enrichment media too.

BA and CA plates were incubated in a CO2 jar at 37°C. BHI and Thio agar will be incubated at 37°C.

A positive culture was defined as growth on any one of the 4 media used.

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BA and CA plates were incubated in a CO2 jar at 37°C. BHI and Thio agar will be incubated at 37°C.

A positive culture was defined as growth on any one of the 4 media used.

8. Colony count was carried out on all positive cultures, classified as light growth (1ess than 20 colonies), moderate growth (20 - 100 colonies), and heavy growth (more than 100 colonies)

9. If no growth was obtained the plates were incubated for another 24 hrs. In case of negative cultures, the plates were then discarded. In case of negative growth on solid media, smears were made directly from the broth that showed turbidity (which is an indication of growth). Subcultures were made from both the broths.

10. Identification of the bacteria were made using standard bacteriological methods.

11. All the micro organisms were tested to antibiotics chosen on the basis of the gram stain results. The Kirby Bauer disc diffusion method of sensitivity testing was applied.[38]

The PI (Principal Investigator) intervenes at the level of reviewing the questionnaires and data processing and analysis.

5.9 ETHICAL CONSIDERATIONS

1. Confidentiality of the patients' records was observed
2. Consent and permission were sought from the Department of Ophthalmology to access the data records.
3. The Consent form utilized to collect data from 1994 to 1997 shown in appendix IV.

5.10 STUDY LIMITATIONS

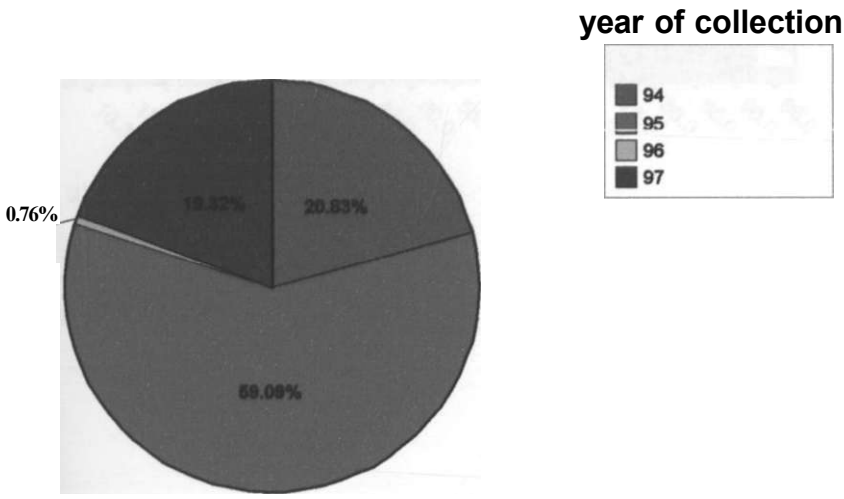
1. The PI had no control over the case selection or the data distribution
2. The PI had no influence on the study protocol that was utilized to obtain the data in 1994 to 1997

6.1 RESULTS ON CONJUNCTIVAL NORMAL FLORA AMONGST KENYANS

TABLE 1: DISTRIBUTION OF DATA AS PER YEAR OF COLLECTION (n=264)

Year	cases	%
1994	55	20.8%
1995	156	59.1%
1996	2	.8%
1997	51	19.3%
Total	264	100.0%

FIGURE 1s DISTRIBUTION OF DATA AS PER YEAR OF COLLECTION (n=264)

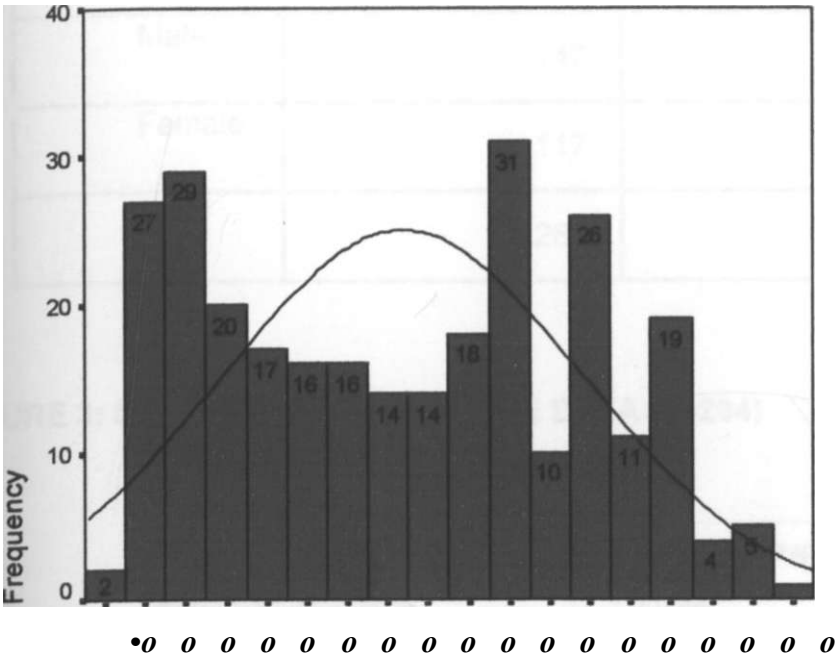


The pie chart shows the distribution of the data by the year of collection. 59.1% of the data was collected in '95 from KEU. The study protocol in both KEU and KNH was the same

* <A>,

DISTRIBUTION

FIGURE 2: AGE DISTRIBUTION OF THE SUBJECTS



Median = 46.5 yrs

Std deviation = 22.29 yrs

Range 11 -95 yrs

The age distribution was skewed towards the right with 2 peaks occurring at the 3rd and the 6th decades. The modal age being in the 6th decade.

TABLE 2: SEX DISTRIBUTION OF VOLUNTEERS '94 TO '97 (n=264)

Sex	Cases	%
Male	147	55.7%
Female	117	44.3%
Total	264	100.0%

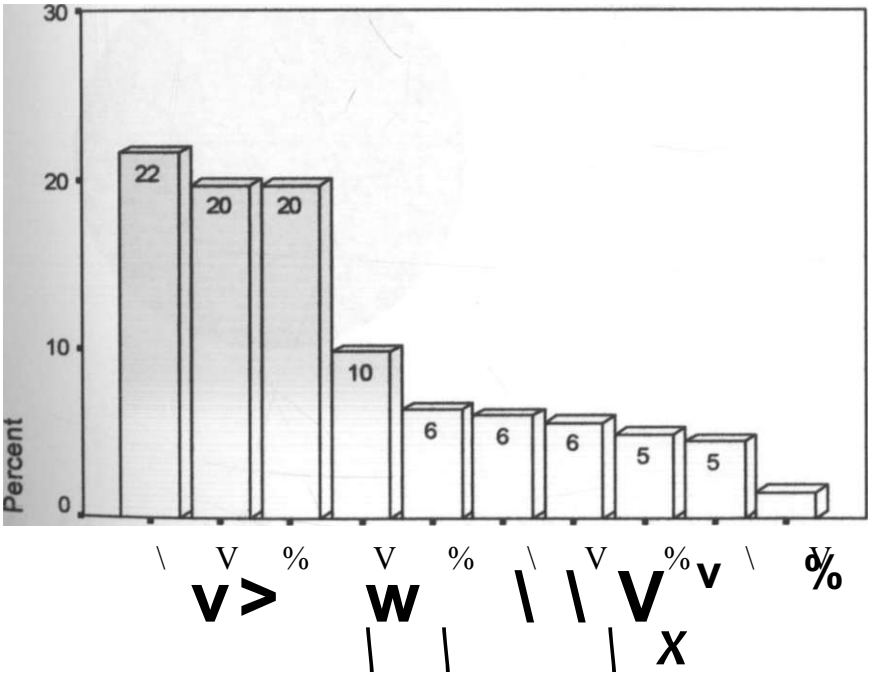
FIGURE 3: SEX DISTRIBUTION OF THE DATA (n=264)

The sex of the study population was evenly distributed (44.32% females and 55 .68% males) (P=0.409) The ratio of male to female is 5:4

TABLE 3: SEX DISTRIBUTION OF VOLUNTEERS '94 TO '97 (n=264)

Occupation	cases	%
housewife	57	21.6%
student	52	19.7%
retired	52	19.7%
casual laborer	26	9.8%
others	17	6.4%
hospital worker	16	6.1%
self employed	15	5.7%
secretary	13	4.9%
farmer	12	4.5%
teacher	4	1.5%
Total	264	100.0%

FIGURE 4: OCCUPATION OF VOLUNTEERS (n=264)



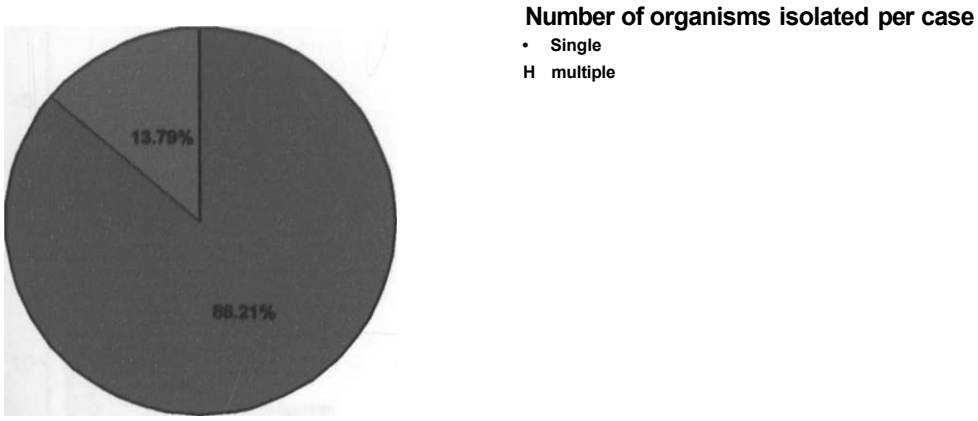
occupation

ost of the volunteers were housewives followed by students and then by retired/farmer male Patients.

TABLE 4: SINGLE VERSUS MIXED ORGANISMS (n=116)

Organisms isolated	Cases	%
Single	100	86.2%
Mixed	16	13.8%
Total	116	100.0%

FIGURE 5: SINGLE VS MIXED ORGANISMS ISOLATED (n=116)

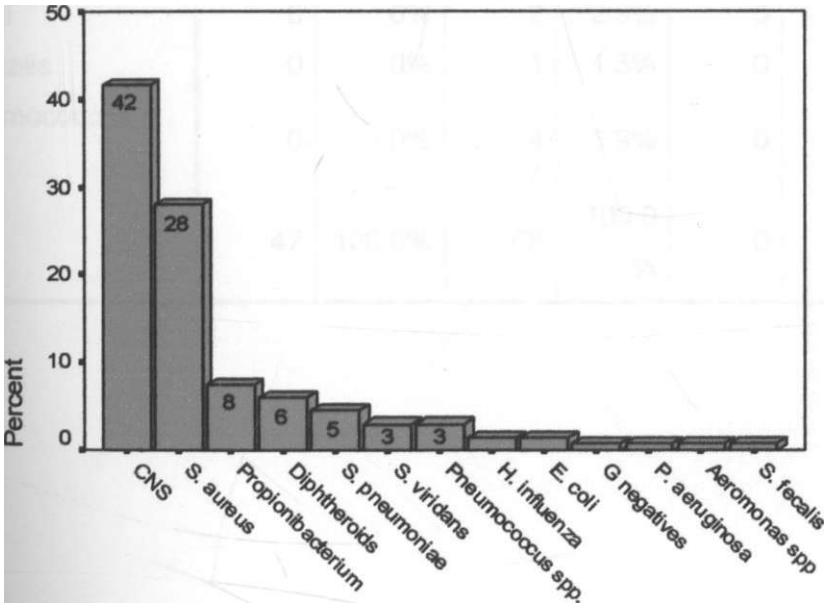


13.8% of the cases had 2 organisms isolated from a single swab inoculation. None had more than 2 organisms isolated in this review.

TABLE 5: ORGANISMS ISOLATED FROM '94 TO *97(n=132)

Organisms Isolated	Count	%
CNS	55	41.7%
S. aureus	37	28.0%
Propionibacterium	10	7.6%
Diphtheroids	8	6.1%
S. pneumoniae	6	4.5%
S. viridans	4	3.0%
Pneumococcus spp.	4	3.0%
H. influenza	2	1.5%
E. coli	2	1.5%
G negatives	1	.8%
P. aeruginosa	1	.8%
Aeromonas spp	1	.8%
S. fecalis	1	.8%
Total	132	100.0%

FIGURE 6: ORGANISMS ISOLATED FROM '94 TO '97 (n=132)

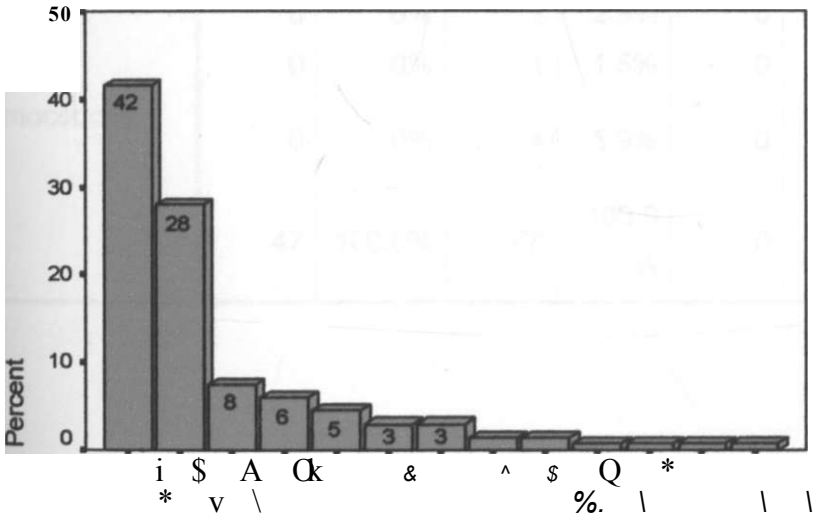


A total of 132 cases were positive from the 264 volunteers. CNS was the most isolated organism accounting for 41.7%, S.aureus was 28%, Propionibacterium 7.6% and Diphtheroids 6.1%. Our [da]*a is similar to data from other regions of the world

TABLE 41: ORGANISMS ISOLATED FROM '94 TO *97(n=132)

Organisms Isolated	Count	%
CNS	55	41.7%
S. aureus	37	28.0%
Propionibacterium	10	7.6%
Diphtheroids	8	6.1%
S. pneumoniae	6	4.5%
S. viridans	4	3.0%
Pneumococcus spp.	4	3.0%
H. influenza	2	1.5%
E. coli	2	1.5%
G negatives	1	8
P. aeruginosa	1	.8%
Aeromonas spp	1	.8%
S. fecalis	1	.8%
Total	132	100.0%

FIGURE 6: ORGANISMS ISOLATED FROM '94 TO '97 (n=132)



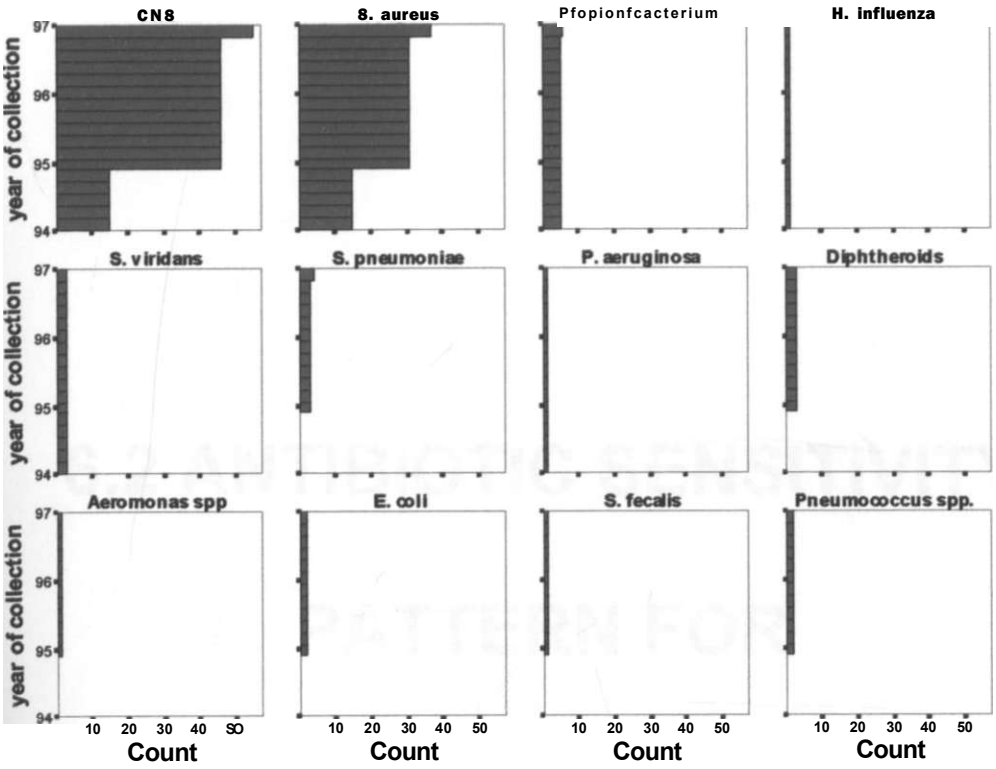
organisms

A total of 132 cases were positive from the 264 volunteers. CNS was the most isolated organism accounting for 41.7%, S.aureus was 28%, Propionibacterium 7.6% and Diphtheroids 6.1%. Our data is similar to data from other regions of the world

TABLE 6: DISTRIBUTION OF ORGANISMS AS PER YEAR OF COLLECTION(n=132)

	year of collection							
	94		95		96		97	
	Count	%	Count	%	Count	%	Count	%
CNS	15	31.9%	31	45.6%	0	.0%	9	52.9%
S. aureus	15	31.9%	16	23.5%	0	.0%	6	35.3%
propionibacterium	9	19.1%	0	.0%	0	.0%	1	5.9%
G negatives	1	2.1%	0	.0%	0	.0%	0	.0%
H. influenza	1	2.1%	1	1.5%	0	.0%	0	.0%
S. viridans	4	8.5%	0	.0%	0	.0%	0	.0%
S. pneumoniae	0	.0%	5	7.4%	0	.0%	1	5.9%
P. aeruginosa	1	2.1%	0	.0%	0	.0%	0	.0%
Diphtheroids	1	2.1%	7	10.3%	0	.0%	0	.0%
Aeromonas spp	0	.0%	1	1.5%	0	.0%	0	.0%
E. coli	0	.0%	2	2.9%	0	.0%	0	.0%
S. fecalis	0	.0%	1	1.5%	0	.0%	0	.0%
Pneumococcus spp.	0	.0%	4	5.9%	0	.0%	0	.0%
Total	47	100.0%	68	100.0 %	0	.0%	17	100.0%

FIGURE 7: DISTRIBUTION OF ORGANISMS AS PER YEAR OF COLLECTION(n=132)



P=0.126 change of flora as per year of collection is statistically insignificant. Pattern of flora does not change with the passage of time. Other studies have shown that with the introduction of foreign materials to the eye, the normal flora pattern remains the same, but there is an increase in the quantity of organisms inhabiting the conjunctiva.

6.2 ANTIBIOTIC SENSITIVITY

PATTERN FOR

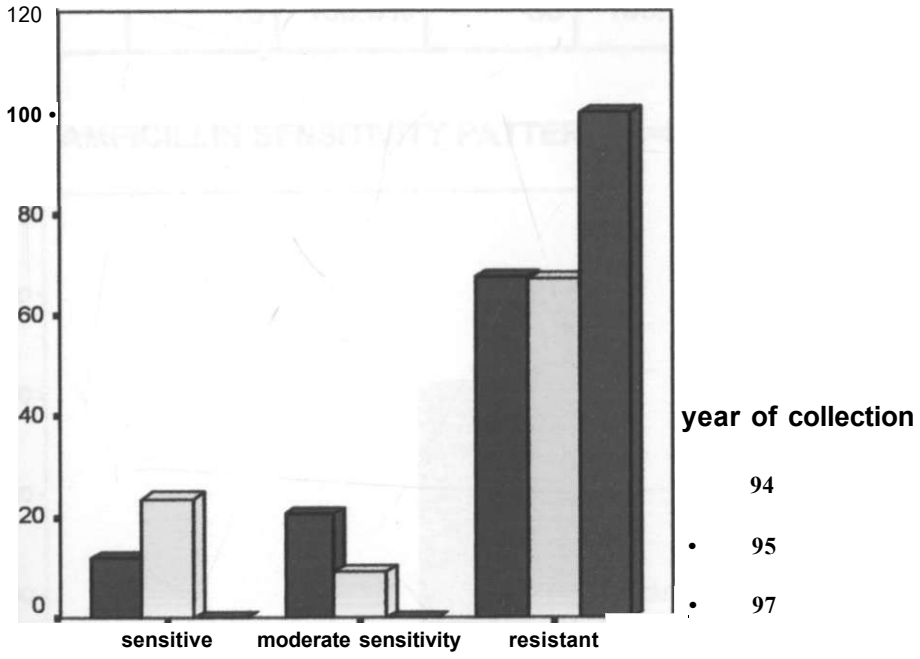
Coagulase

Negative Staphylococci

TABLE 7:CHLORAMPHENICOL SENSITIVITY PATTERN (n=26)

Amoxicillin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
Sensitive	3	21.4%	5	16.7%	0	.0%
moderate sensitive	3	21.4%	4	13.3%	0	.0%
Resistant	8	57.1%	21	70.0%	8	100.0%
Total	14	100.0%	30	100.0%	8	100.0%

FIGURE 8: AMOXICILLIN SENSITIVITY PATTERN (n=52)



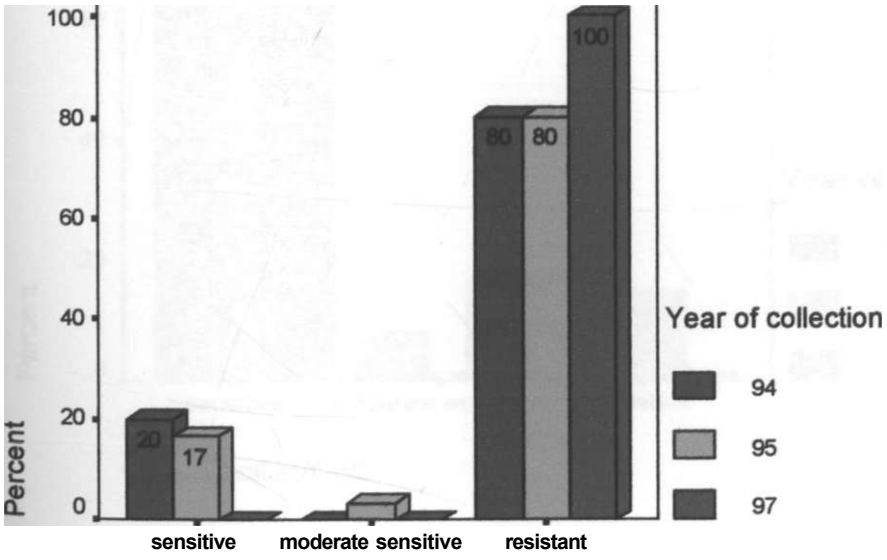
.324 The resistance of amoxicillin increased overall with 100% resistance in 1997

TABLE 8: CARBENICILLIN SENSITIVITY PATTERN (n=30)

Ampicillin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
sensitive	3	20.0%	5	16.7%	0	.0%
moderate sensitive	0	.0%	1	3.3%	0	.0%
resistant	12	80.0%	24	80.0%	8	100.0%
Total	15	100.0%	30	100.0%	8	100.0%

FIGURE 9: AMPICILLIN SENSITIVITY PATTERN (n=53)

120



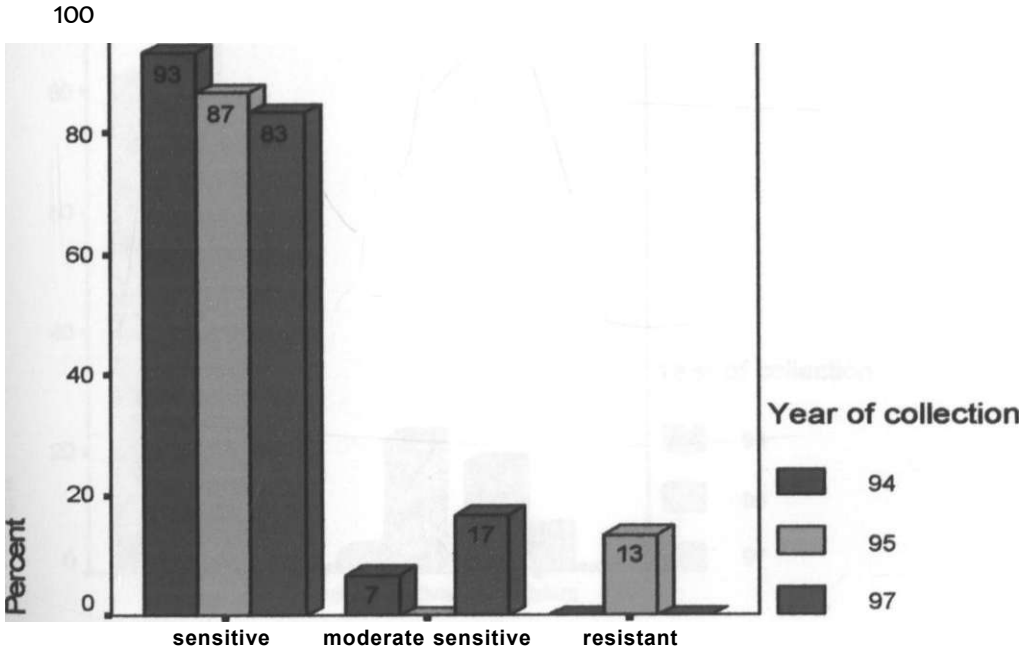
Ampicillin/CNS

**=0.431 The resistance remained high with an increase to 100% in 1997

TABLE 9: CARBENICIILIN SENSITIVITY PATTERN (n=51)

Carbenicillin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
sensitive	14	93.3%	26	86.7%	5	83.3%
moderate sensitive	1	6.7%	0	.0%	1	16.7%
resistant	0	.0%	4	13.3%	0	.0%
Total	15	100.0%	30	100.0%	6	100.0%

FIGURE 10: CARBENICILLIN SENSITIVITY PATTERN (n=51)

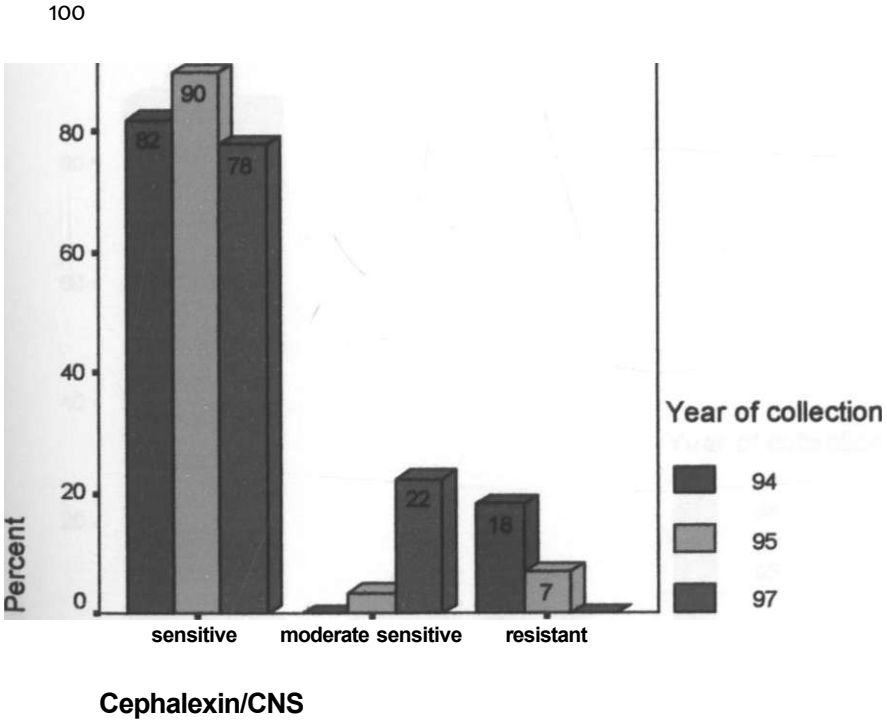


The sensitivity to Carbenicillin remained high. Although there is a mild drop in the sensitivity, it is statistically insignificant P=0.146

TABLE 10: CEPHALEXIN SENSITIVITY PATTERN (n=49)

Cephalexin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
sensitive	9	81.8%	26	89.7%	7	77.8%
moderate sensitive	0	.0%	1	3.4%	2	22.2%
resistant	2	18.2%	2	6.9%	0	.0%
Total	11	100.0%	29	100.0%	9	100.0%

FIGURE 11: CEPHALEXIN SENSITIVITY PATTERN (n=49)

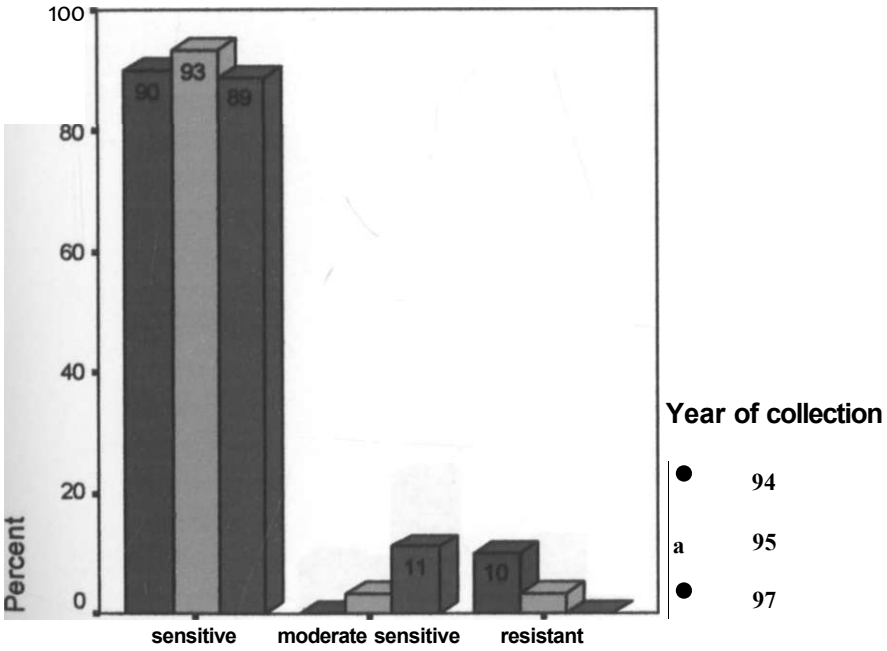


The sensitivity of Cephalexin **P**=0.130 remained good overall.

TABLE 11:CHLORAMPHENICOL SENSITIVITY PATTERN (n=26)

Cefalotin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
sensitive	9	90.0%	28	93.3%	8	88.9%
moderate sensitive	0	.0%	1	3.3%	1	11.1%
resistant	1	10.0%	1	3.3%	0	.0%
Total	10	100.0%	30	100.0%	9	100.0%

FIGURE 12: CEFALOTIN SENSITIVITY PATTERN (n=49)



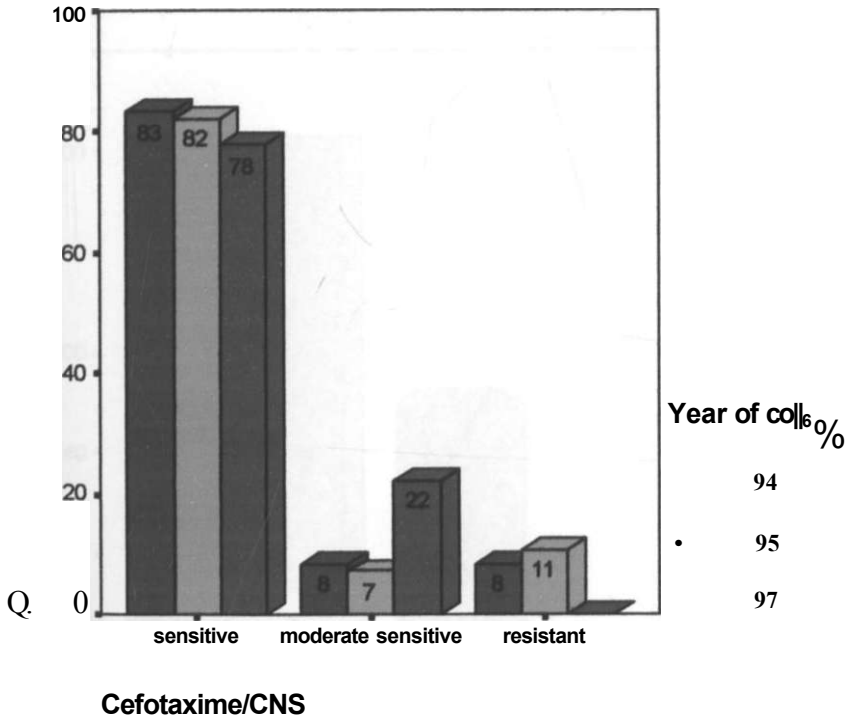
The sensitivity of the antibiotic remained high all through. (P=0.588)

TABLE 12:CHLORAMPHENICOL SENSITIVITY PATTERN (n=26)

Cefotaxime	Year of collection				
	94		95		
	Count	%	Count	%	e
sensitive	10	83.3%	23	82.1%	
moderate sensitive	1	8.3%	2	7.1%	
resistant	1	8.3%	3	10.7%	
Total	12	100.0%	28	100.0%	

77 - 8%

FIGURE 13: CEFOTAXIME SENSITIVITY PATTERN (n=49)

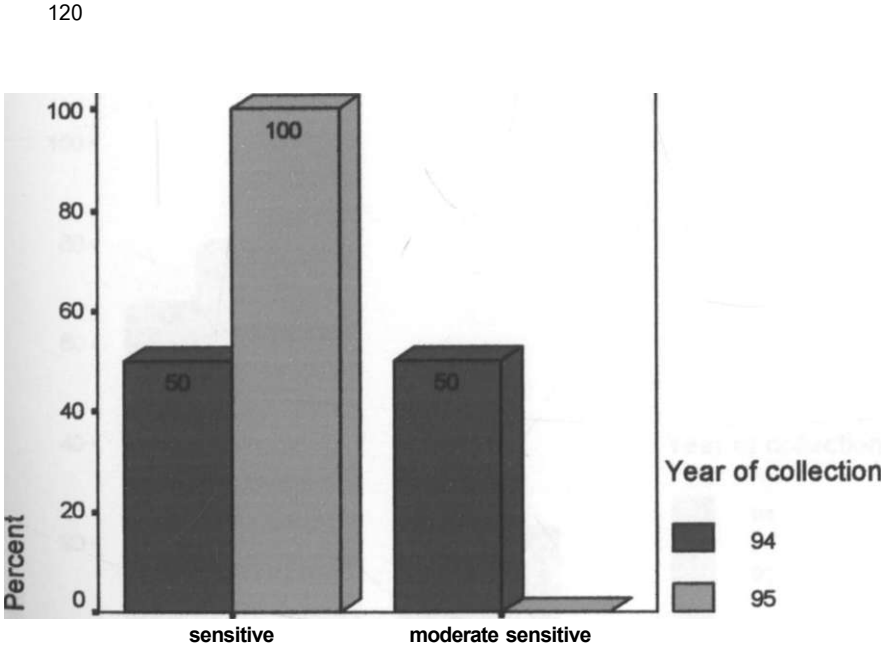


The sensitivity of Cefotaxime to CNS was good all through (P=0.63^

TABLE 13:CEFTAZIDIME SENSITIVITY PATTERN (n=5)

Ceftazidime	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
sensitive	1	50.0%	3	100.0%	0	.0%
moderate sensitive	1	50.0%	0	.0%	0	.0%
resistant	0	0%	0	0%	0	0%
Total	2	100.0%	3	100.0%	0	.0%

FIGURE 14: CEFTAZIDIME SENSITIVITY PATTERN (n=5)



Ceftazidime/CNS

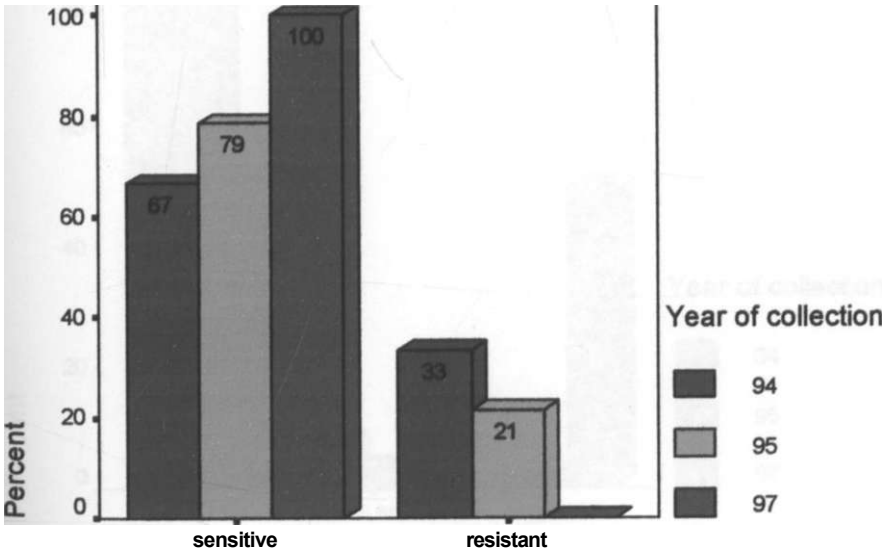
The sensitivity remained high although the number of cases tested against ceftazidime was low, with no sensitivity tests for 1997. (P=0.400). The resistance pattern is not shown as there was no data.

TABLE ^CHLORAMPHENICOL SENSITIVITY PATTERN (n=46)

Chloramphenicol	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
sensitive	10	66.7%	22	78.6%	3	100.0%
resistant	5	33.3%	6	21.4%	0	.0%
Total	15	100.0%	28	100.0%	3	100.0%

FIGURE 15: CHLORAMPHENICOL SENSITIVITY PATTERN (n=46)

120 r



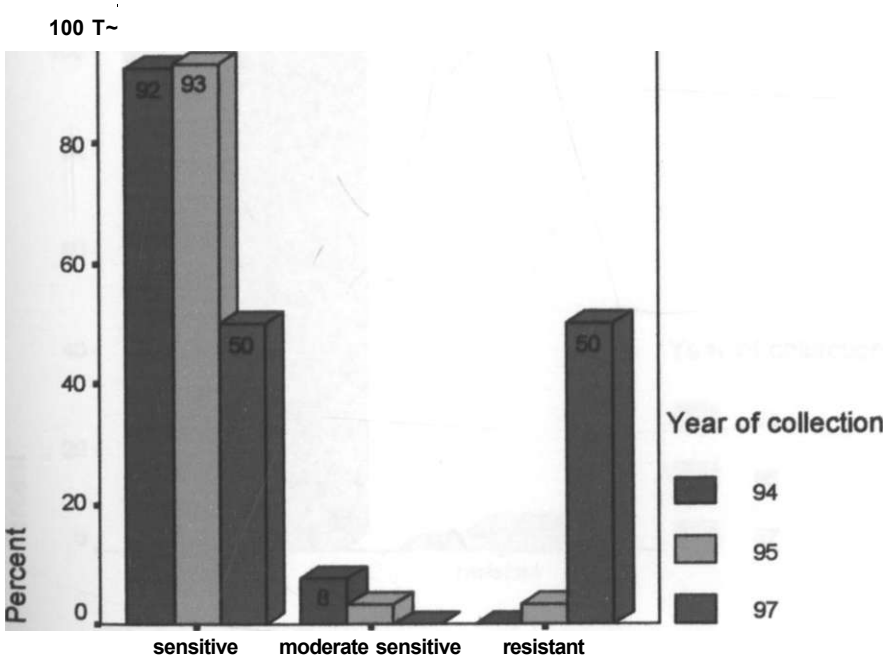
Chloramphenicol/CNS

There was an overall increase in the sensitivity, with **100%** sensitivity in **1997.** There were no cases **for** moderate sensitivity all through **(P=0.413).** No graph for moderate sensitivity since no data available.

TABLE 15:CIPR0FL0XACIN SENSITIVITY PATTERN (n=44)

Ciprofloxacin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
sensitive	12	92.3%	27	93.1%	1	50.0%
moderate sensitive	1	7.7%	1	3.4%	0	.0%
resistant	0	.0%	1	3.4%	1	50.0%
Total	13	100.0%	29	100.0%	2	100.0%

FIGURE 16: CIPROFLOXACIN SENSITIVITY PATTERN (n=44)



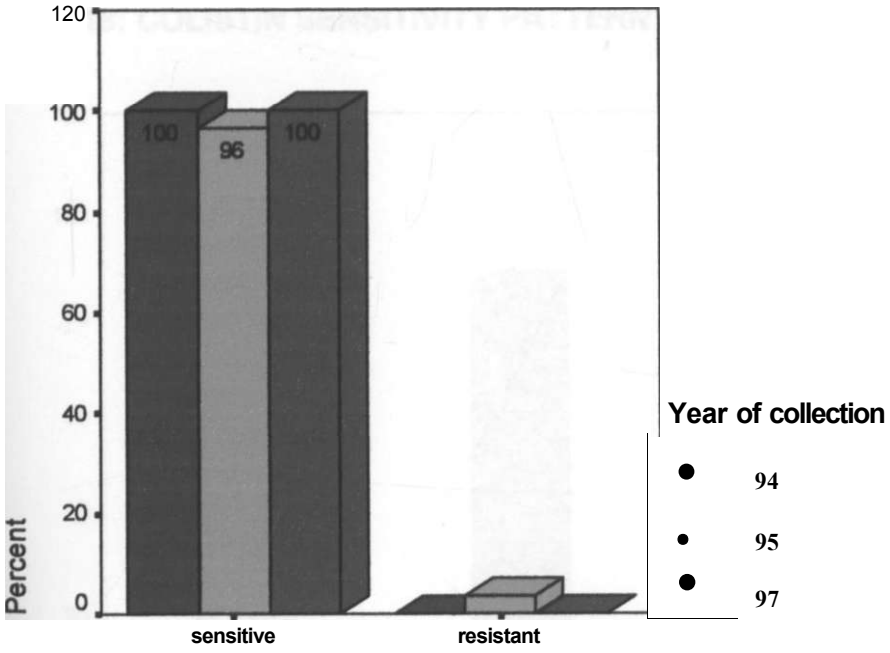
Ciprofloxacin/CNS

There is an increase in the resistance to ciprofloxacin, the number of cases examined to **were** only 2 in 1997. (P=0.032)

TABLE 16:CHLORAMPHENICOL SENSITIVITY PATTERN (n=26)

Clindamycin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
sensitive	1	100.0%	27	96.4%	9	100.0%
Moderate sensitivity	0	0%	0	0%	0	0%
resistant	0	.0%	1	3.6%	0	.0%
Total	1	100.0%	28	100.0%	9	100.0%

FIGURE 17: CLINDAMYCIN SENSITIVITY PATTERN (n=38)



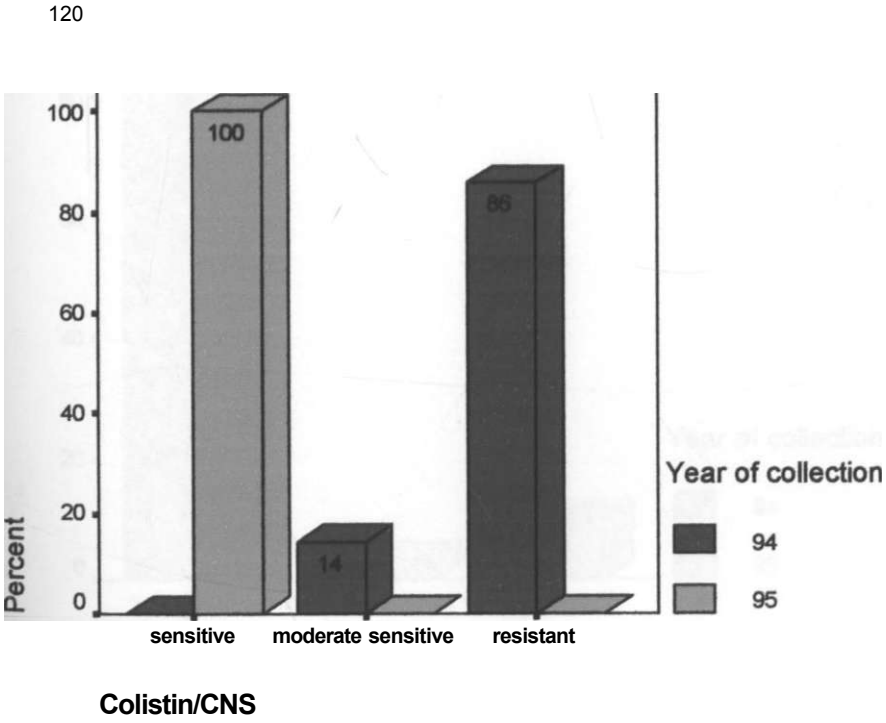
Clindarrycin/CNS

Clindamycin remained highly sensitive to CNS (P=0.832)

TABLE 17: TRIMETHOPRIM SENSITIVITY PATTERN (n=16)

Colistin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
sensitive	0	.0%	1	100.0%	0	.0%
moderate sensitive	1	14.3%	0	.0%	0	.0%
resistant	6	85.7%	0	.0%	0	.0%
Total	7	100.0%	1	100.0%	0	.0%

FIGURE 18: COLISTIN SENSITIVITY PATTERN (n=8)

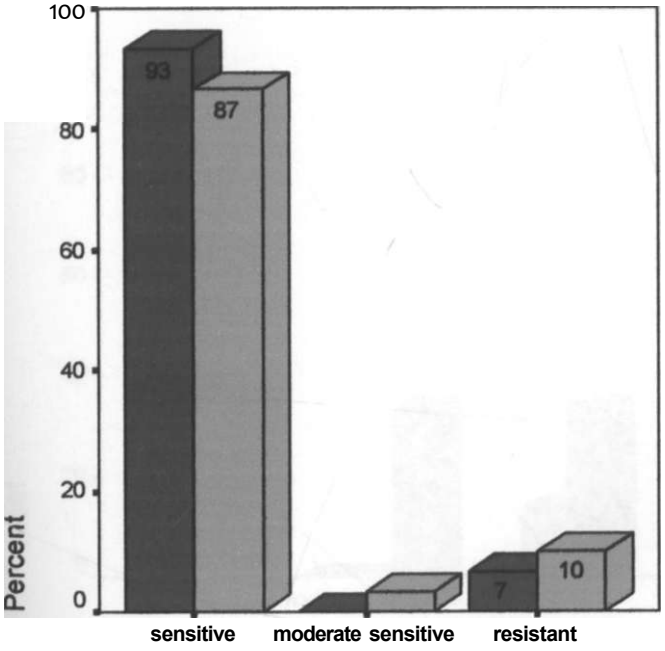


There was a statistically significant increase in the sensitivity of colistin to CNS. The antibiotic was not tested against CNS in 1997 (P=0.018).

TABLE 18:CHLORAMPHENICOL SENSITIVITY PATTERN (n=26)

Erythromycin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
Sensitive	14	93.3%	26	86.7%	0	.0%
moderate sensitive	0	.0%	1	3.3%	0	.0%
Resistant	1	6.7%	3	10.0%	0	.0%
Total	15	100.0%	30	100.0%	0	.0%

FIGURE 19: ERYTHROMYCIN SENSITIVITY PATTERN (n=45)



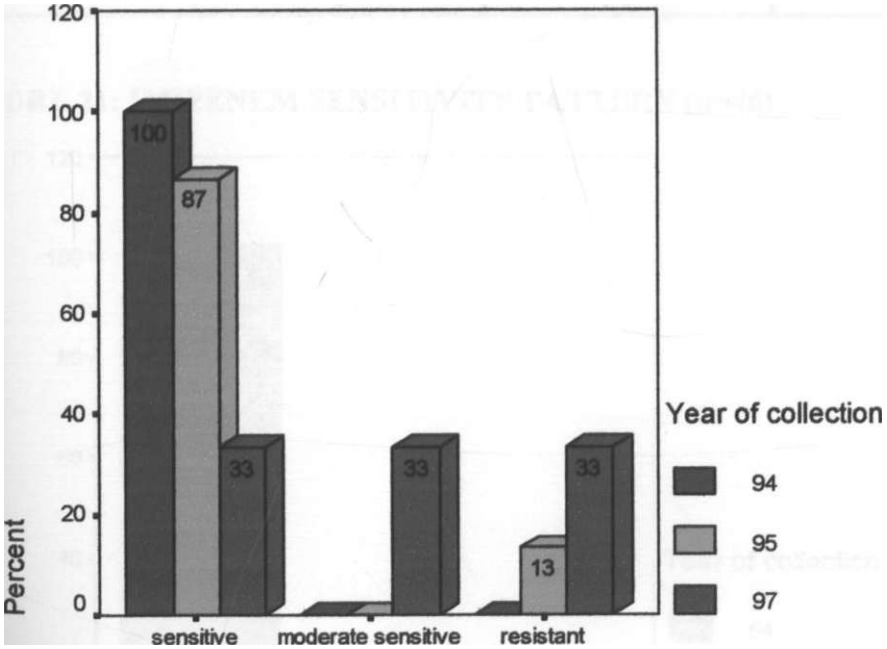
My⁰/*

There were no sensitivity patterns available for 1997. Erythromycin was highly sensitive to CNS. (P=0.714)

TABLE 19:CHLORAMPHENICOL SENSITIVITY PATTERN (n=26)

Gentamicin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
Sensitive	10	100.0%	26	86.7%	1	33.3%
moderate sensitive	0	.0%	0	.0%	1	33.3%
Resistant	0	.0%	4	13.3%	1	33.3%
Total	10	100.0%	30	100.0%	3	100.0%

FIGURE 20: GENTAMICIN SENSITIVITY PATTERN (n=43)



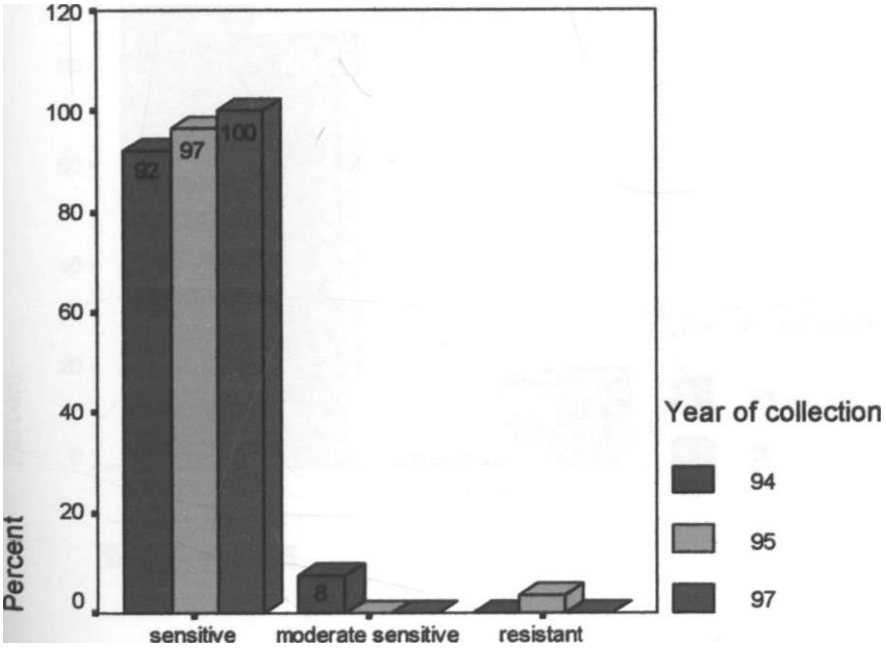
Gentamicin/CNS

There is **a** statistically significant increase in the resistance to gentamicin over the years **(P**=0.002)

TABLE 20: NORFLOXACIN SENSITIVITY PATTERN (n=28)

Imipenem	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
Sensitive	12	92.3%	28	96.6%	4	100.0%
moderate sensitive	1	7.7%	0	.0%	0	.0%
Resistant	0	.0%	1	3.4%	0	.0%
Total	13	100.0%	29	100.0%	4	100.0%

FIGURE 21: IMIPENEM SENSITIVITY PATTERN (n=46)



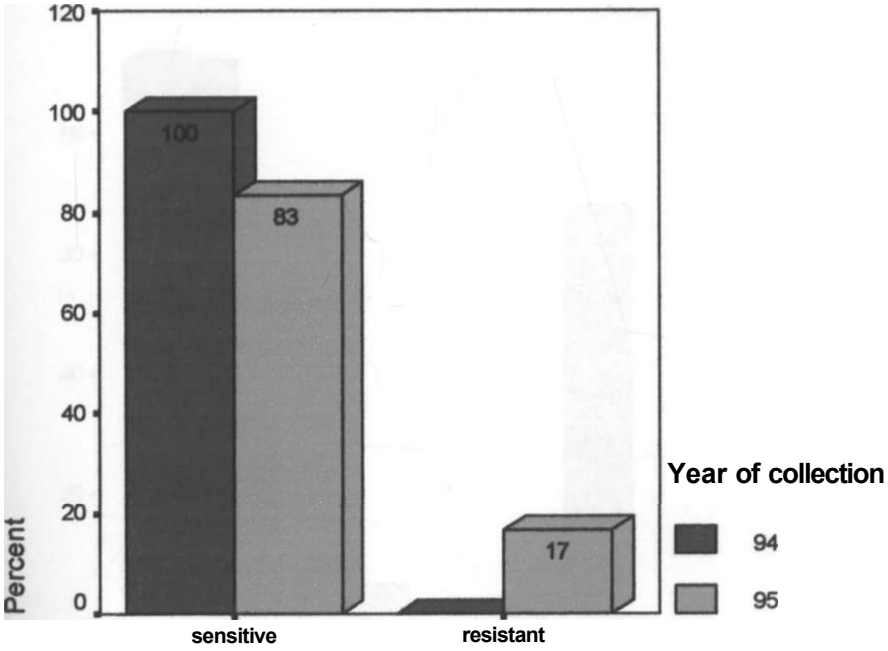
ImipenenrVCNS

Imipenem remained highly sensitive to CNS **over the years.** There **was a gradual increase in the sensitivity over the years (P=0.533)**

TABLE 21:CHLORAMPHENICOL SENSITIVITY PATTERN (n=26)

Kanamycin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
sensitive	13	100.0%	25	83.3%	0	.0%
Moderate sensitivity	0	0%	0	0%	0	0%
resistant	0	.0%	5	16.7%	0	.0%
Total	13	100.0%	30	100.0%	0	.0%

FIGURE 22: KANAMYCIN SENSITIVITY PATTERN (n=43)

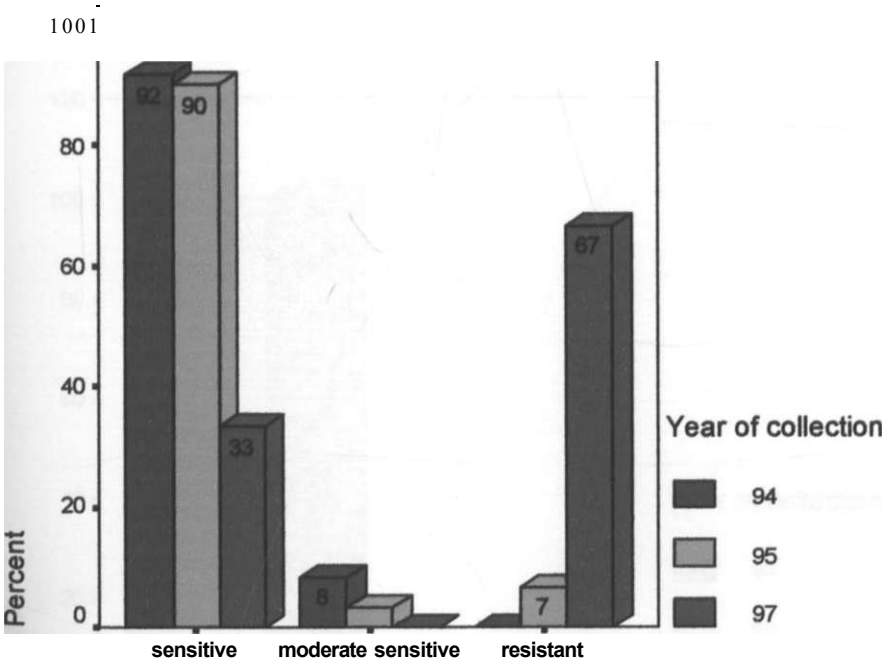


There was a mild increase in the resistance of Kanamycin to CNS although it remained a good antibiotic although (P=0.148)

TABLE 22: NORFLOXACIN SENSITIVITY PATTERN (n=45)

Norfloxacin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
Sensitive	11	91.7%	27	90.0%	1	33.3%
moderate sensitive	1	8.3%	1	3.3%	0	.0%
Resistant	0	.0%	2	6.7%	2	66.7%
Total	12	100.0%	30	100.0%	3	100.0%

FIGURE 23: NORFLOXACIN SENSITIVITY PATTERN (n=45)

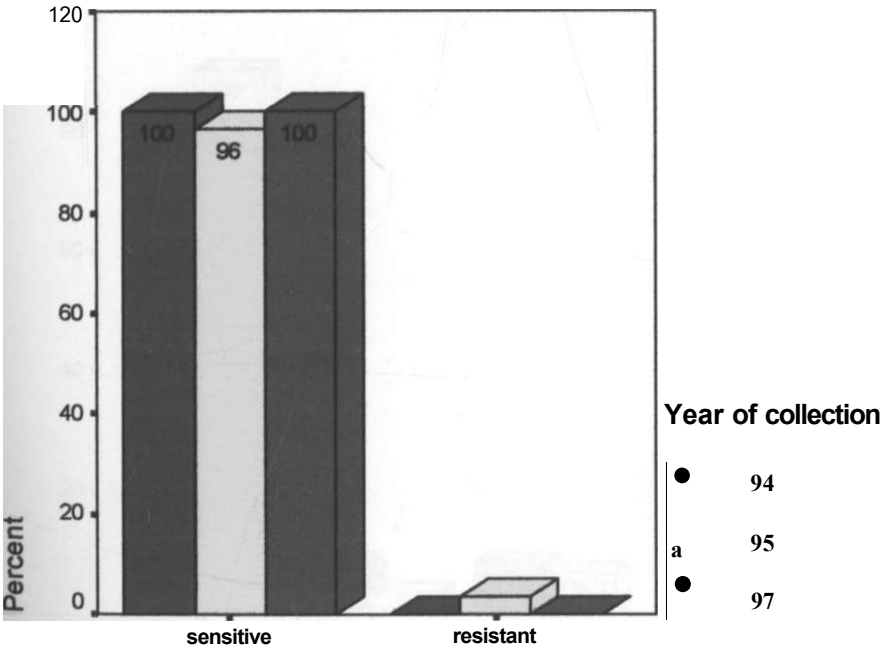


There was a statistically significant increase in the resistance of the antibiotic over the years (P=0.007)

TABLE 23:CIPR0FL0XACIN SENSITIVITY PATTERN (n=44)

Ofloxacin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
sensitive	11	100.0%	27	96.4%	1	100.0%
Moderate sensitivity	0	0%	0	0%	0	0%
resistant	0	.0%	1	3.6%	0	.0%
Total	11	100.0%	28	100.0%	1	100.0%

FIGURE 24: OFLOXACIN SENSITIVITY PATTERN (n=40)



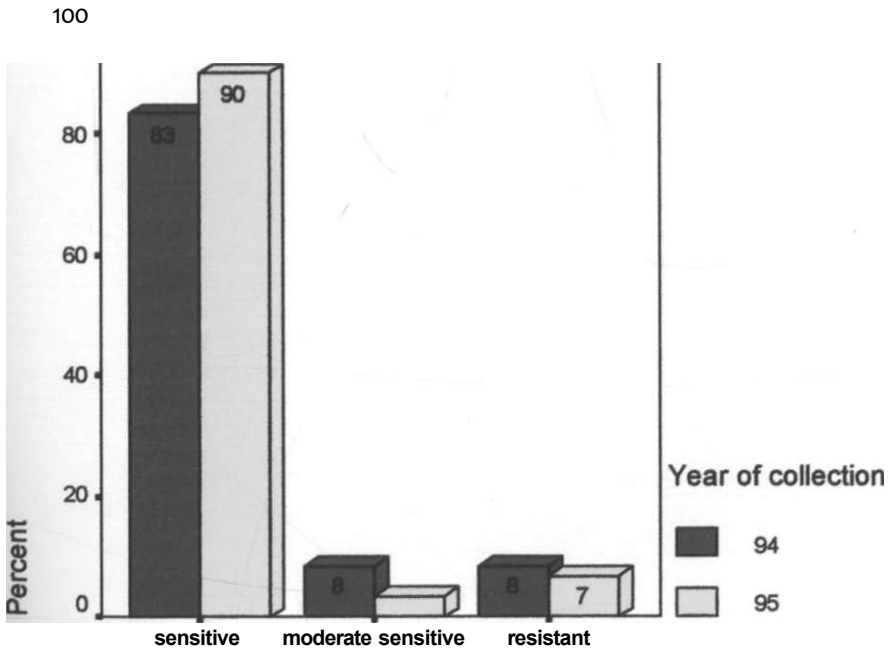
Ofloxacin/CNS

Ofloxacin was highly sensitive to CNS over the years (P=0.803)

TABLE 24: POLYMIXIN B SENSITIVTY PATTERN (n=42)

Polymixin B	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
Sensitive	10	83.3%	27	90.0%	0	.0%
moderate sensitive	1	8.3%	1	3.3%	0	.0%
Resistant	1	8.3%	2	6.7%	0	.0%
Total	12	100.0%	30	100.0%	0	.0%

FIGURE 25: POLYMIXIN B SENSITIVITY PATTERN (n=42)



Polymixin B/CNS

There was no data collected for 1997. The antibiotic remained highly sensitive overall (P=0.769)

TABLE 25: TRIMETHOPRIM SENSITIVITY PATTERN (n=16)

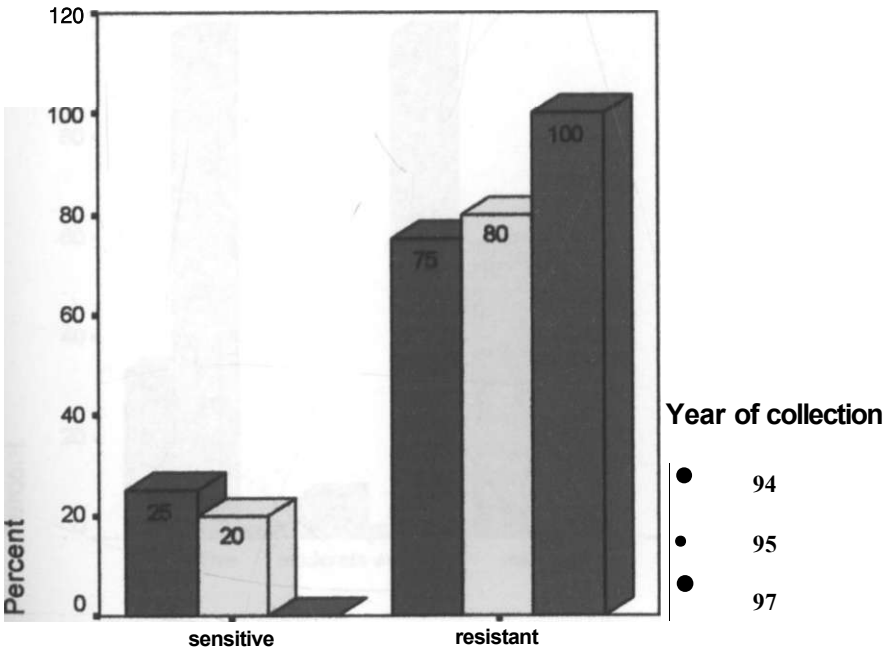
Penicillin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
sensitive	0	0%	0	0%	0	0%
Moderate sensitivity	0	0%	0	0%	0	0%
resistant	1	100.0%	0	.0%	0	.0%
Total	1	100.0%	0	.0%	0	.0%

There was 1 case tested and this was resistant to penicillin

TABLE 26:CIPR0FL0XACIN SENSITIVITY PATTERN (n=44)

Tetracycline	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
sensitive	3	25.0%	6	20.0%	0	.0%
Moderate sensitivity	0	0%	0	0%	0	0%
resistant	9	75.0%	24	80.0%	1	100.0%
Total	12	100.0%	30	100.0%	1	100.0%

FIGURE 26: TETRACYCLINE SENSITIVITY PATTERN (n=43)



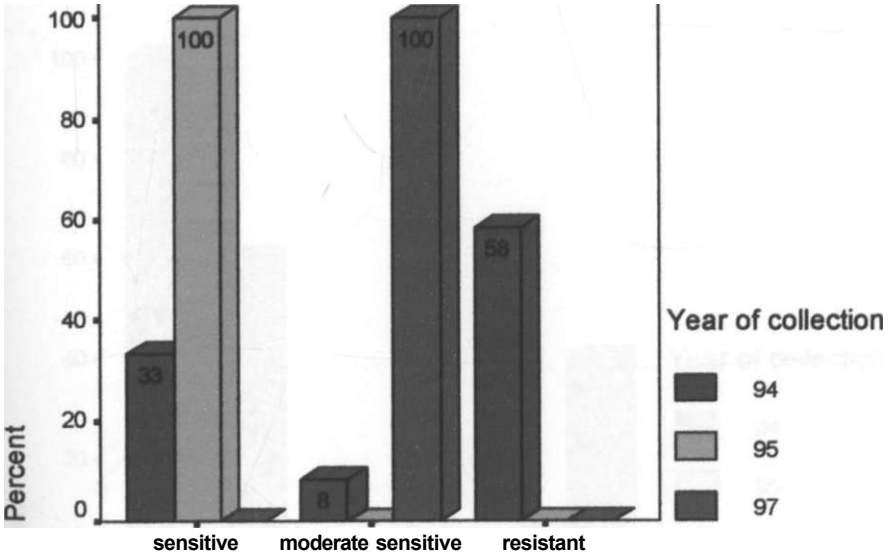
Tetracycline was highly resistant all through and the resistance steadily increased over the years (P=0.819)

TABLE 27: TRIMETHOPRIM SENSITIVITY PATTERN (n=16)

Trimethoprim	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
Sensitive	4	33.3%	2	100.0%	0	.0%
moderate sensitive	1	8.3%	0	.0%	2	100.0%
Resistant	7	58.3%	0	.0%	0	.0%
Total	12	100.0%	2	100.0%	2	100.0%

FIGURE 27: TRIMETHOPRIM SENSITIVITY PATTERN (n=16)

120



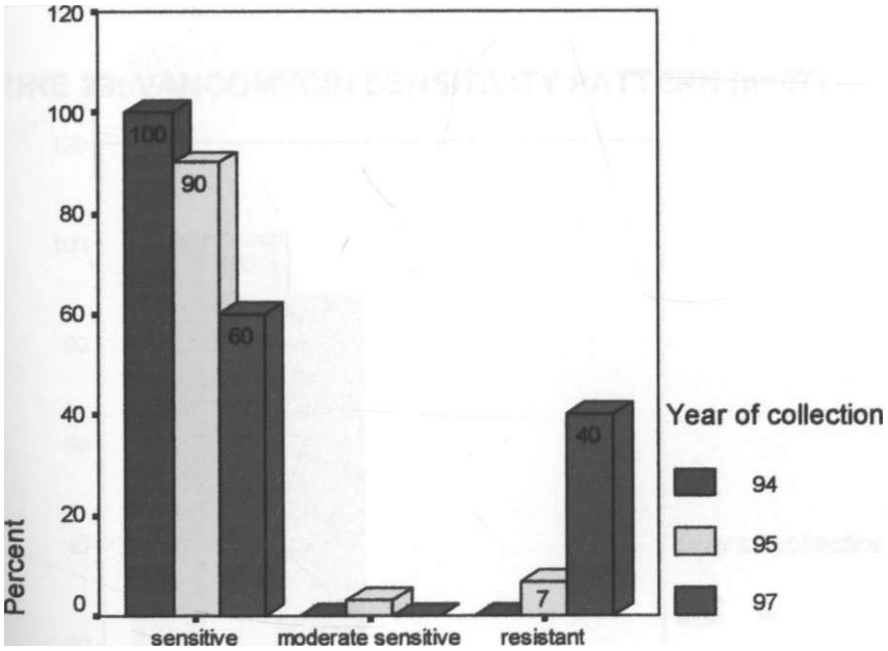
trimethoprim/CNS

There was a statistically significant decrease in the sensitivity of the antibiotic over the years (P=0.01)

TABLE 28: TOBRAMYCIN SENSITIVTY PATTERN (n=47)

Tobramycin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
Sensitive	12	100.0%	27	90.0%	3	60.0%
moderate sensitive	0	.0%	1	3.3%	0	.0%
Resistant	0	.0%	2	6.7%	2	40.0%
Total	12	100.0%	30	100.0%	5	100.0%

FIGURE 28: TOBRAMYCIN SENSITIVITY PATTERN (n=47)



Tobramycin/CNS

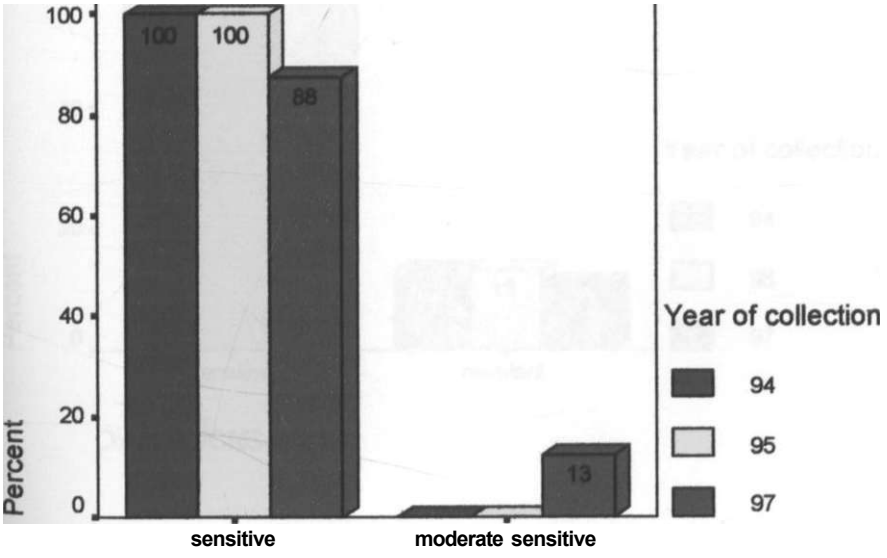
There was a gradual increase in the resistance of the antibiotic over the years (P=0.086)

TABLE 29: NORFLOXACIN SENSITIVITY PATTERN (n=28)

Vancomycin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
Sensitive	9	100.0%	30	100.0%	7	87.5%
moderate sensitive	0	.0%	0	.0%	1	12.5%
resistant	0	0%	0	0%	0	0%
Total	9	100.0%	30	100.0%	8	100.0%

FIGURE 29: VANCOMYCIN SENSITIVITY PATTERN (n=47)

120



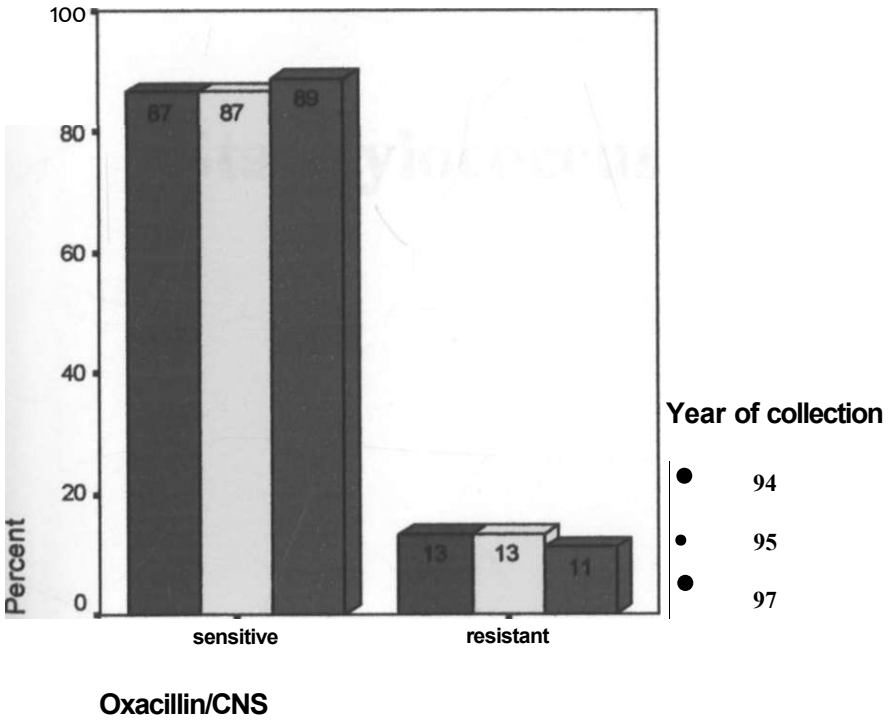
Vancomycin/CNS

Vancomycin remained highly sensitive all through the years (P=0.083)

TABLE 30:CHLORAMPHENICOL SENSITIVITY PATTERN (n=26)

Oxacillin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
sensitive	13	86.7%	26	86.7%	8	88.9%
moderate sensitivity	0	0%	0	0%	0	0%
resistant	2	13.3%	4	13.3%	1	11.1%
Total	15	100.0%	30	100.0%	9	100.0%

FIGURE 30: OXACILLIN SENSITIVITY PATTERN (n=54)



Oxacillin remained sensitive all through P=0.984

.3ANTIBIOTIC SENSITIVITY

PATTERN FOR

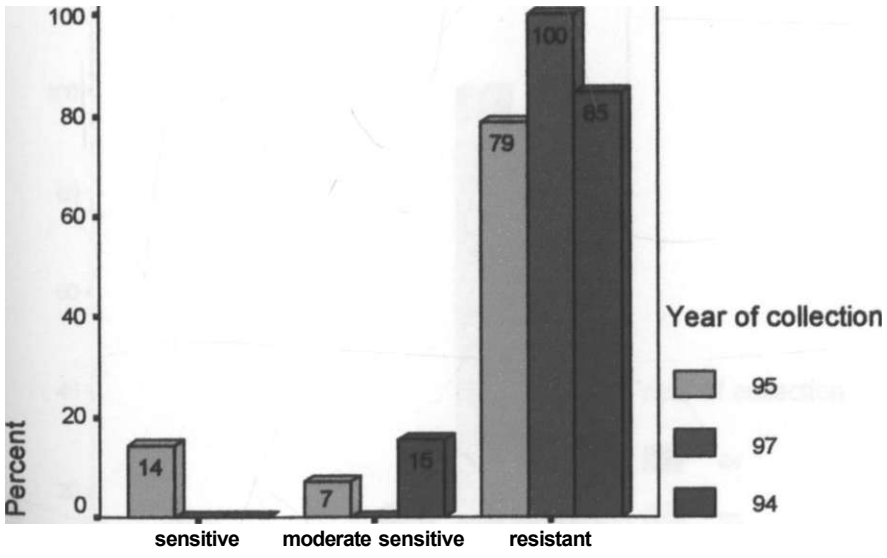
Staphylococcus aureus

TABLE 31: AMOXICILLIN SENSITIVITY PATERN (n=32)

Amoxicillin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
Sensitive	0	.0%	2	14.3%	0	.0%
moderate sensitive	2	15.4%	1	7.1%	0	.0%
Resistant	11	84.6%	11	78.6%	5	100.0%
Total	13	100.0%	14	100.0%	5	100.0%

FIGURE 31: AMOXICILLIN SENSITIVITY PATTERN (n=32)

1201



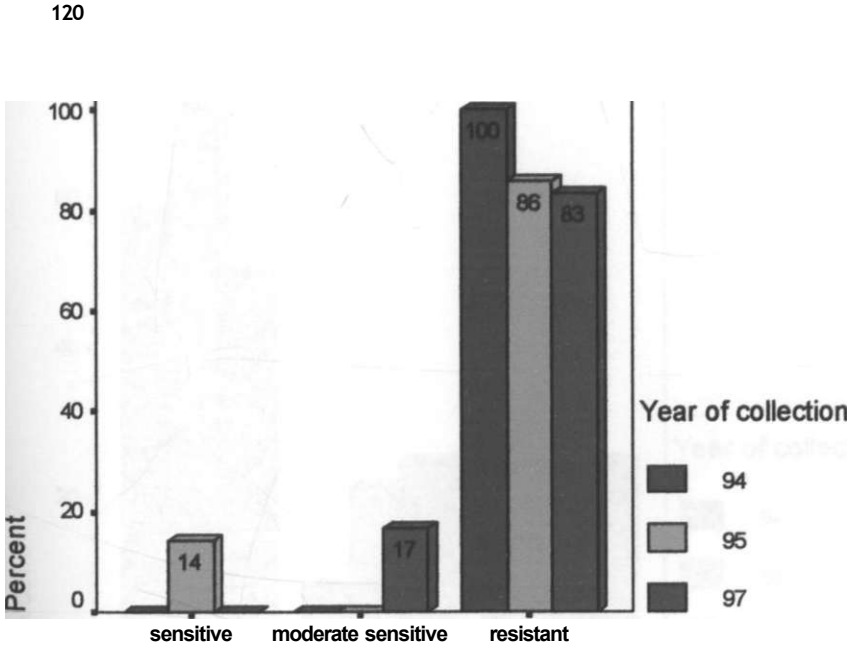
Amoxicillin/S.aureus

Amoxicillin had been highly resistant all through to S.aureus P=0.431

TABLE 32:CHLORAMPHENICOL SENSITIVITY PATTERN (n=26)

Ampicillin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
Sensitive	0	.0%	2	14.3%	0	.0%
Moderate sensitive	0	.0%	0	.0%	1	16.7%
Resistant	12	100.0%	12	85.7%	5	83.3%
Total	12	100.0%	14	100.0%	6	100.0%

FIGURE 32: AMPICILLIN SENSITIVITY PATTERN (n=32)



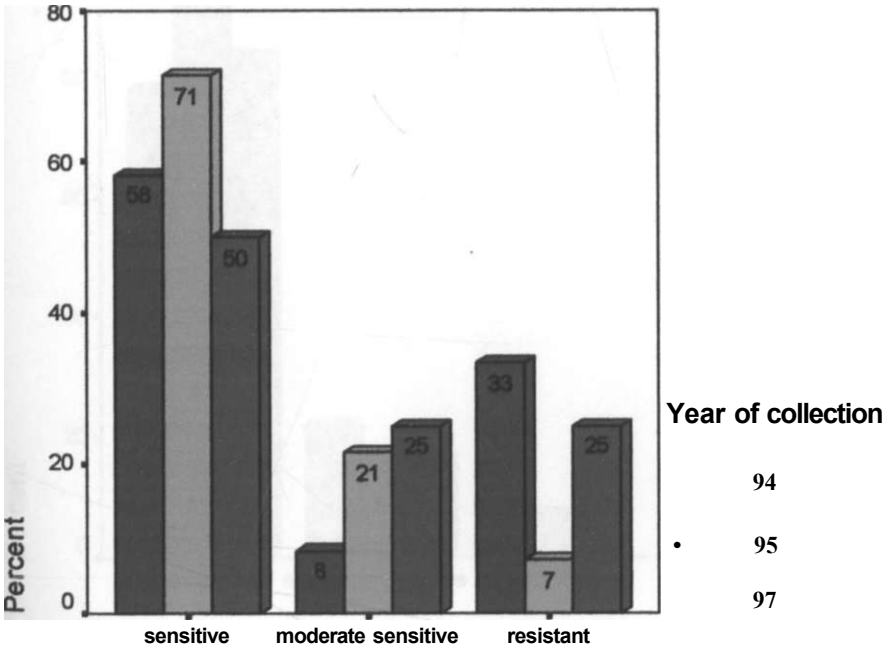
Ampicillin/S.aureus

There was a mild decrease in the resistance of the antibiotic, but it remained a highly resistant antibiotic (P=0.431)

TABLE 33: CARBENIC1LLIN SENSITIVITY PATTERN (n=30)

Carbenicillin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
Sensitive	7	58.3%	10	71.4%	2	50.0%
moderate sensitive	1	8.3%	3	21.4%	1	25.0%
Resistant	4	33.3%	1	7.1%	1	25.0%
Total	12	100.0%	14	100.0%	4	100.0%

FIGURE 33: CARBENICILLIN SENSITIVITY PATTERN (n=30)



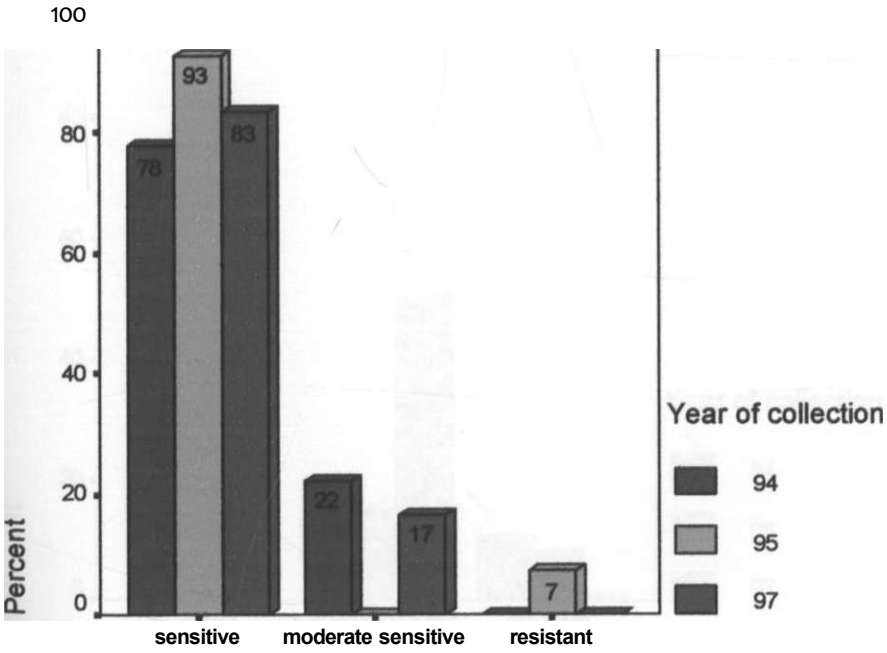
Carbenicillin/S.aureus

S aureus had been resistant to carbenicillin all through **(P=0.488)**

TABLE 34: CEPHALEXIN SENSITIVITY PATTERN (n=29)

Cephalexin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
Sensitive	7	77.8%	13	92.9%	5	83.3%
moderate sensitive	2	22.2%	0	.0%	1	16.7%
Resistant	0	.0%	1	7.1%	0	.0%
Total	9	100.0%	14	100.0%	6	100.0%

FIGURE 34: CEPHALEXIN SENSITIVITY PATTERN (n=29)



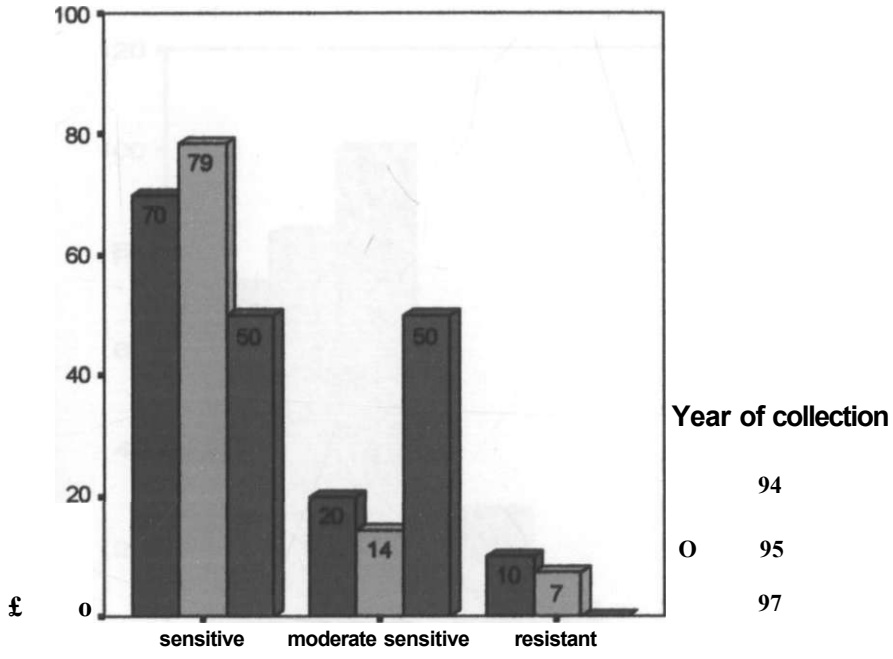
Cephalerin/S.aureus

Cephalexin remained a good antibiotic all through with a good sensitivity pattern (P=0.389)

TABLE 35: NORFLOXACIN SENSITIVITY PATTERN (n=45)

Cefalotin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
Sensitive	7	70.0%	11	78.6%	3	50.0%
moderate sensitive	2	20.0%	2	14.3%	3	50.0%
Resistant	1	10.0%	1	7.1%	0	.0%
Total	10	100.0%	14	100.0%	6	100.0%

FIGURE 35: CEFALOTIN SENSITIVITY PATTERN (n=30)



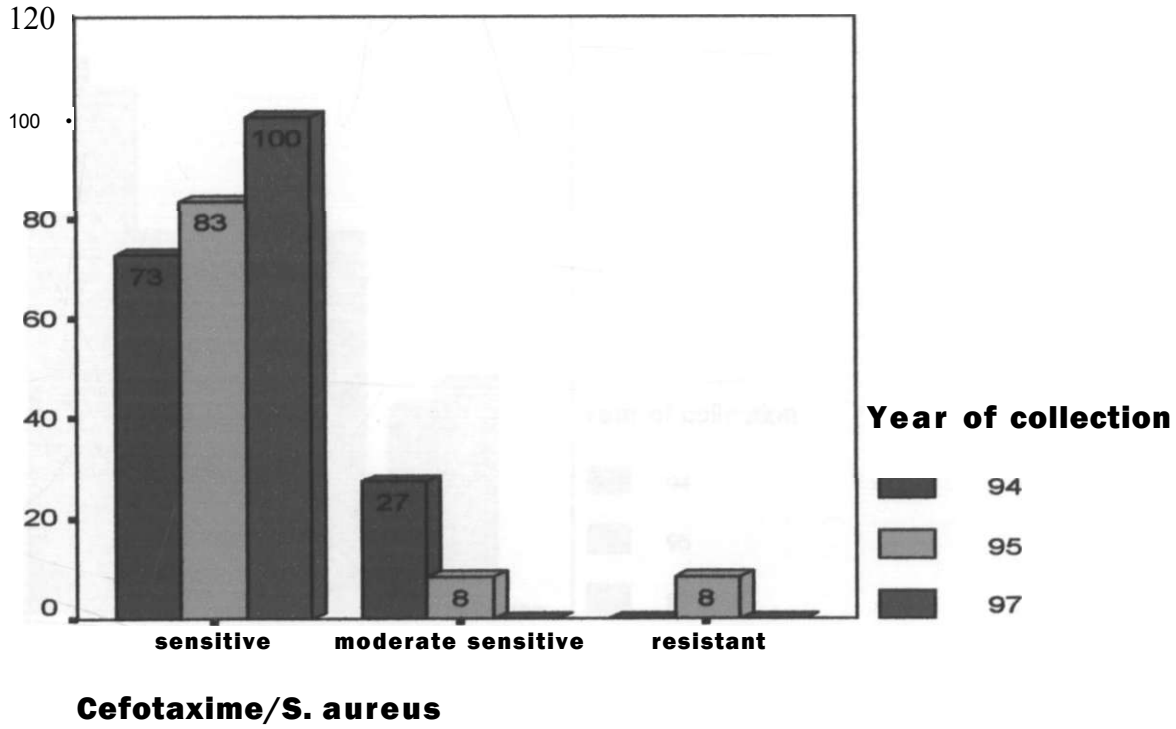
Cefalotin/Saureus

Cefalotin remained a good antibiotic all through with low resistance (P=0.489)

TABLE 36: CEPHALEXIN SENSITIVITY PATTERN (n=49)

Cefotaxime	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
Sensitive	8	72.7%	10	83.3%	5	100.0%
moderate sensitive	3	27.3%	1	8.3%	0	.0%
Resistant	0	.0%	1	8.3%	0	.0%
Total	11	100.0%	12	100.0%	5	100.0%

FIGURE 36: CEFOTAXIME SENSITIVITY PATTERN (n=18)

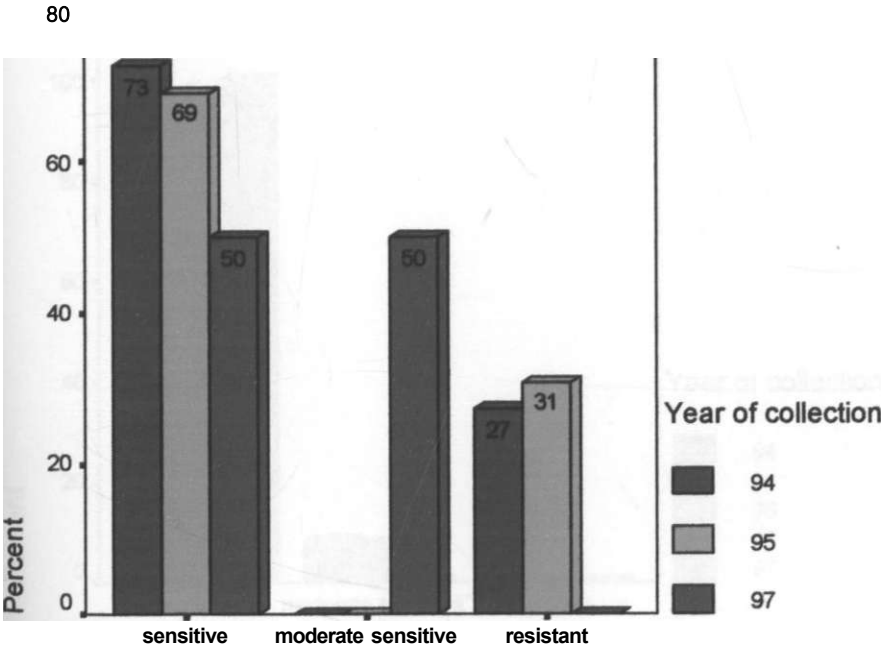


There was a steady increase in the sensitivity of the antibiotic. The antibiotic remained a good antibiotic all through (P=0.412)

TABLE 37:CHLORAMPHENICOL SENSITIVITY PATTERN (n=26)

Chloramphenicol	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
Sensitive	8	72.7%	9	69.2%	1	50.0%
moderate sensitive	0	.0%	0	.0%	1	50.0%
Resistant	3	27.3%	4	30.8%	0	.0%
Total	11	100.0%	13	100.0%	2	100.0%

FIGURE 37: CHLORAMPHENICOL SENSITIVITY PATTERN (n=26)



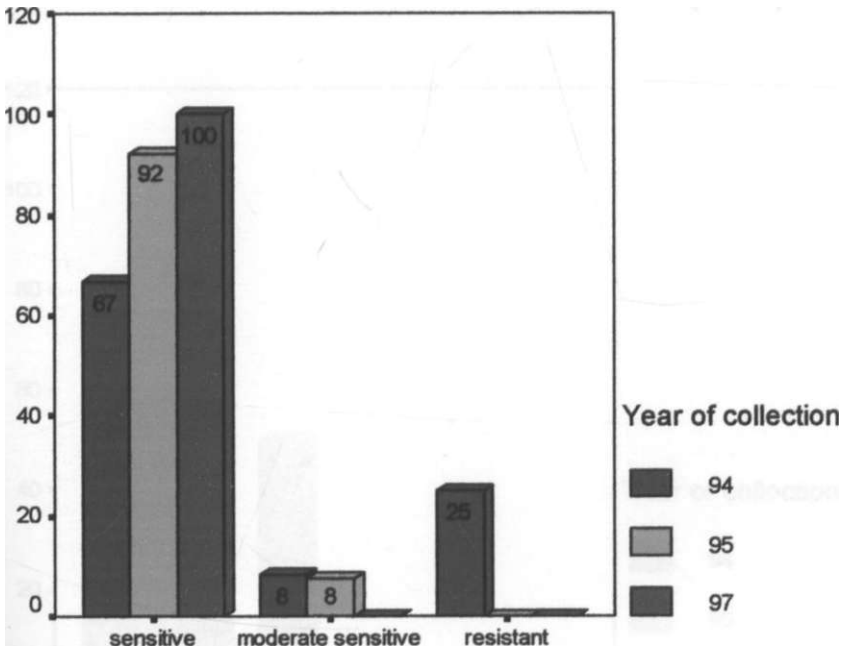
Chloramphenicol/S.aureus

There was a statistically significant fall in the resistance of the antibiotic to S.aureus over the years (JN).013)

TABLE 38:CHLORAMPHENICOL SENSITIVITY PATTERN (n=26)

Ciprofloxacin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
Sensitive	8	66.7%	12	92.3%	4	100.0%
Moderate sensitive	1	8.3%	1	7.7%	0	.0%
Resistant	3	25.0%	0	.0%	0	.0%
Total	12	100.0%	13	100.0%	4	100.0%

FIGURE 38: CIPROFLOXACIN SENSITIVITY PATTERN (n=29)



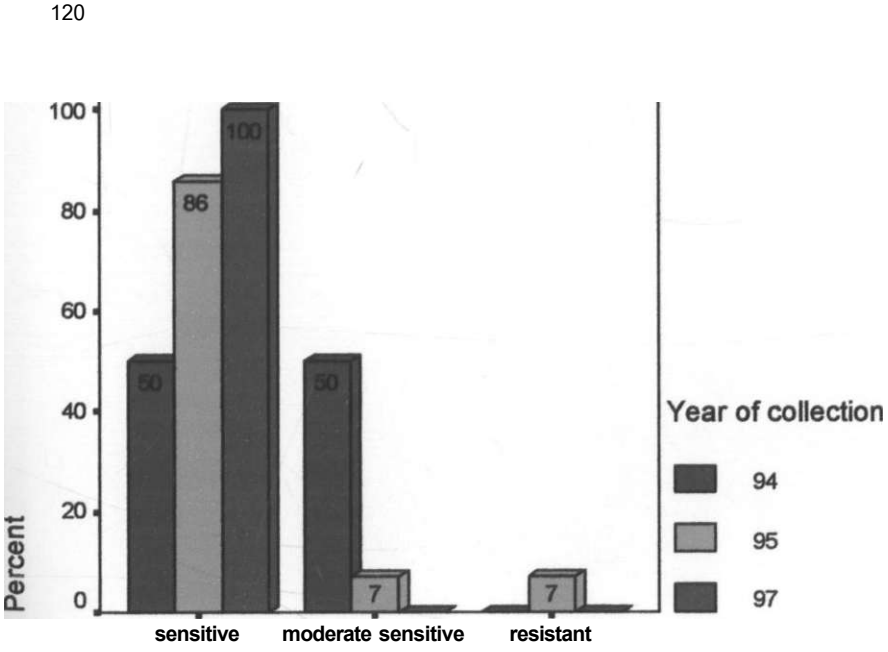
Ciprofloxacin/S.aureus

There was a steady increase in the sensitivity of the antibiotic over the years (P=0.264)

TABLE 39: CEPHALEXIN SENSITIVITY PATTERN (n=49)

Clindamycin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
Sensitive	1	50.0%	12	85.7%	3	100.0%
Moderate sensitive	1	50.0%	1	7.1%	0	.0%
Resistant	0	.0%	1	7.1%	0	.0%
Total	2	100.0%	14	100.0%	3	100.0%

FIGURE 39: CLINDAMYCIN SENSITIVITY PATTERN (n=19)



Clindamycin/S.aureus

There was a steady increase in the sensitivity of the antibiotic over the years to S. aureus. (P=0.385)

TABLE 40-.DOXYCYCLINE SENSITIVITY PATTERN (n=l)

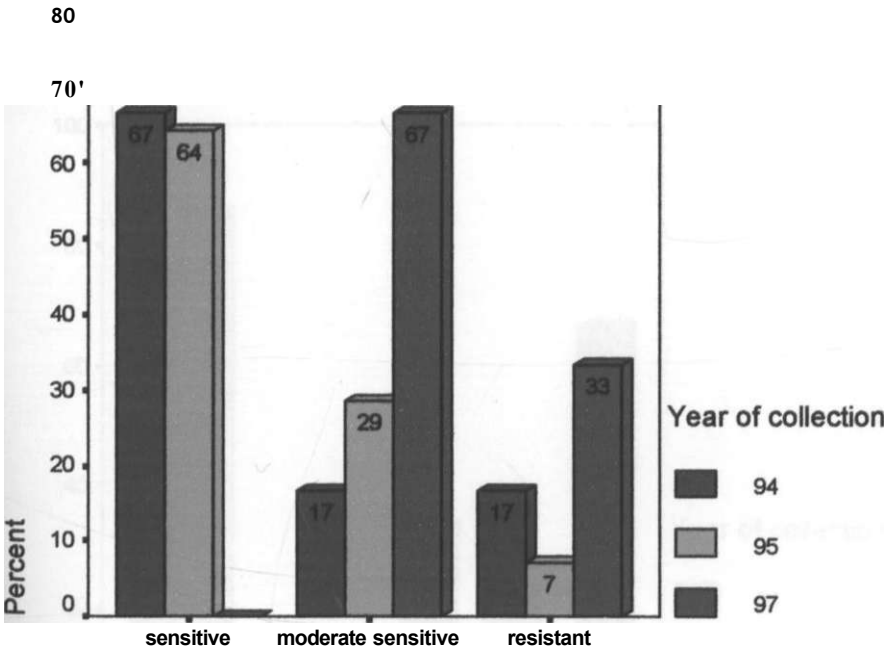
Doxycycline	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
Sensitive	0	0%	0	0%	0	0%
Moderate sensitivity	0	0%	0	0%	0	0%
Resistant	0	.0%	1	100.0%	0	.0%
Total	0	.0%	1	100.0%	0	.0%

There was 1 case of S.aureus tested against doxycycline and this was resistant to Doxycycline

TABLE 41: ERYTHROMYCIN SENSITIVITY PATTERN (n=28)

Erythromycin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
Sensitive	8	66.7%	9	64.3%	0	.0%
Moderate sensitive	2	16.7%	4	28.6%	2	66.7%
Resistant	2	16.7%	1	7.1%	1	33.3%
Total	12	100.0%	14	100.0%	3	100.0%

FIGURE 40: ERYTHROMYCIN SENSITIVITY PATTERN (n=28)

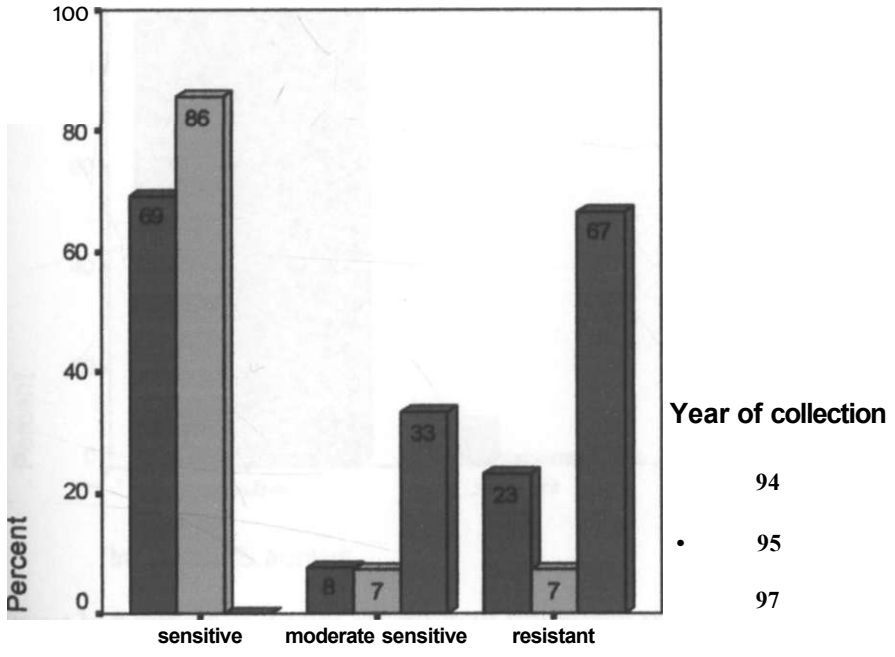


There was an increase in the resistance of S.aureus to Erythromycin over the years **(P**=0.239)

TABLE 42: GENTAMICIN SENSITIVITY PATTERN (n=30)

Gentamicin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
Sensitive	9	69.2%	12	85.7%	0	.0%
Moderate sensitive	1	7.7%	1	7.1%	1	33.3%
Resistant	3	23.1%	1	7.1%	2	66.7%
Total	13	100.0%	14	100.0%	3	100.0%

FIGURE 41: GENTAMICIN SENSITIVITY PATTERN (n=30)

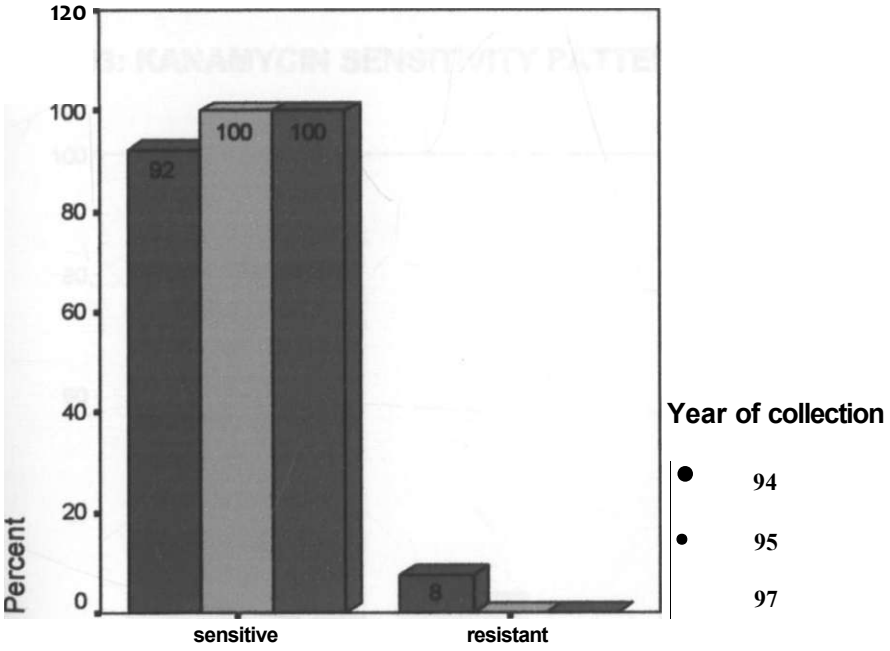


S.aureus had increase in resistance to Gentamicin over the years (P=0.064)

TABLE 43: NORFLOXACIN SENSITIVITY PATTERN (n=45)

Imipenem	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
Sensitive	12	92.3%	14	100.0%	4	100.0%
Moderate sensitivity	0	0%	0	0%	0	0%
Resistant	1	7.7%	0	.0%	0	.0%
Total	13	100.0%	14	100.0%	4	100.0%

FIGURE 42: IMIPENEM SENSITIVITY PATTERN (n=31)



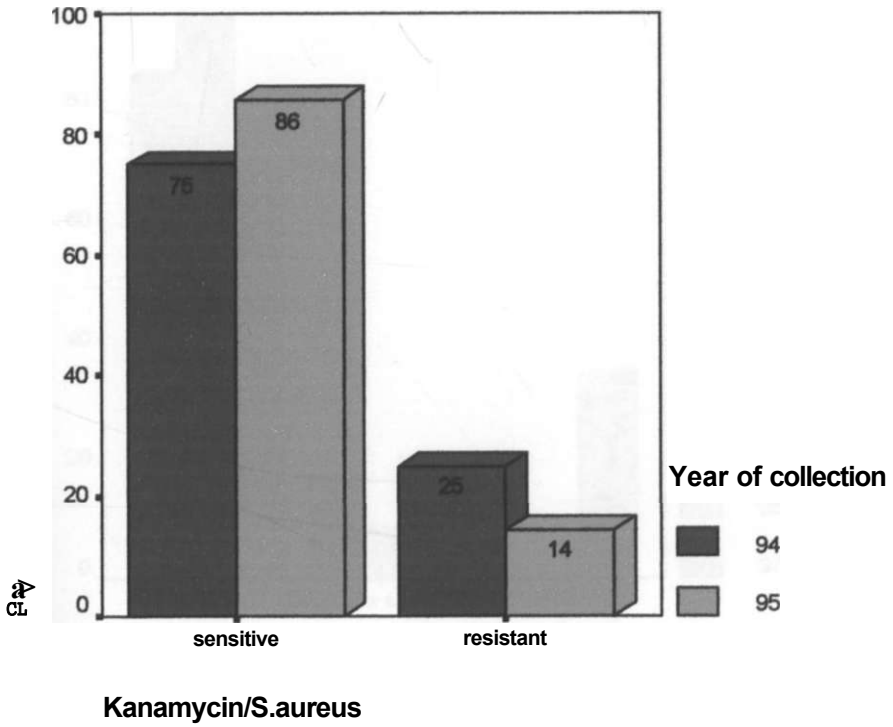
InripenerrVS.aureus

S.aureus had remained highly sensitive to Imipenem over the years (P=0.489)

TABLE 44: NORFLOXACIN SENSITIVITY PATTERN (n=28)

Kanamyacin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
Sensitive	9	75.0%	12	85.7%	0	.0%
Moderate sensitivity	0	0%	0	0%	0	0%
Resistant	3	25.0%	2	14.3%	0	.0%
Total	12	100.0%	14	100.0%	0	.0%

FIGURE 43: KANAMYCIN SENSITIVITY PATTERN (n=26)

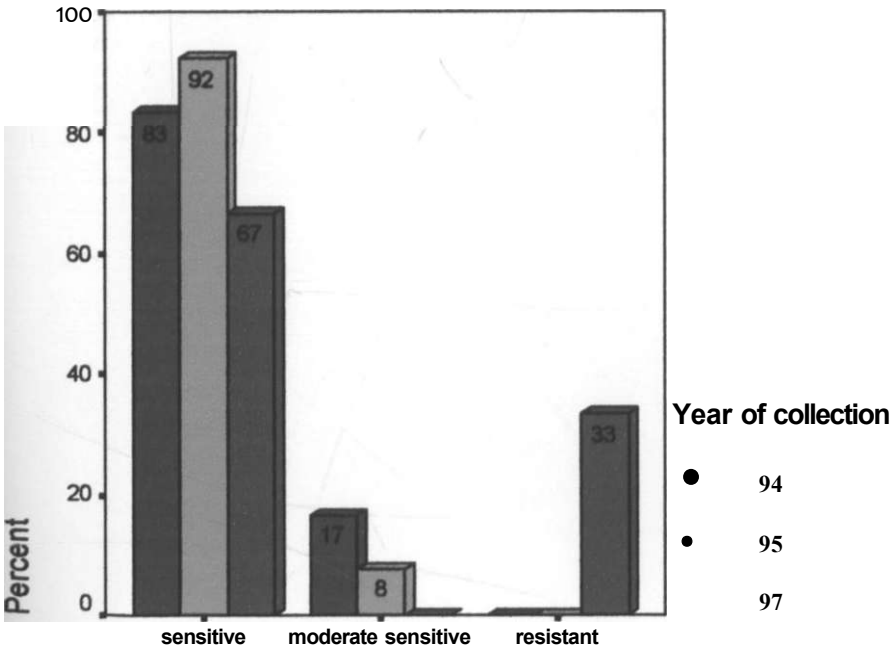


S.aureus remained sensitive to Kanamycin. There were no results for 1997 (P=0.422)

TABLE 45: NORFLOXACIN SENSITIVITY PATTERN (n=28)

Norfloxacin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
Sensitive	10	83.3%	12	92.3%	2	66.7%
Moderate sensitive	2	16.7%	1	7.7%	0	.0%
Resistant	0	.0%	0	.0%	1	33.3%
Total	12	100.0%	13	100.0%	3	100.0%

FIGURE 44: NORFLOXACIN SENSITIVITY PATTERN (n=28)



Norfloxacin/S. aureus

There was an increase in the resistance of the antibiotic in 1997,however there were only 3 cases tested for the sensitivity. (P=0.053)

TABLE 46: NORFLOXACIN SENSITIVITY PATTERN (n=28)

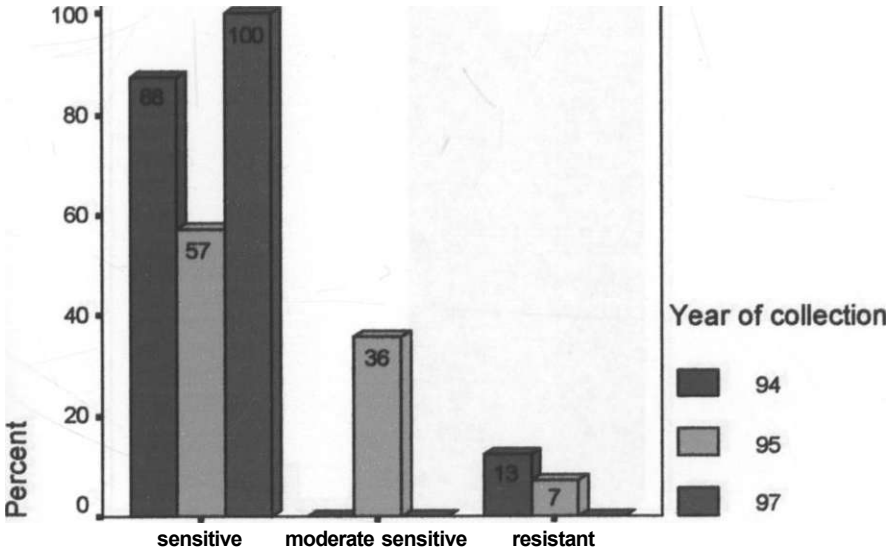
Ofloxacin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
Sensitive	6	100.0%	13	100.0%	1	100.0%
Moderate sensitivity	0	0%	0	0%	0	0%
Resistant	0	0%	0	0%	0	0%
Total	6	100.0%	13	100.0%	1	100.0%

The antibiotic remained 100% sensitive all through the years

TABLE 47: POLYMIXIN B SENSITIVTY PATTERN (n=23)

Polymixin B	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
Sensitive	7	87.5%	8	57.1%	1	100.0%
Moderate sensitive	0	.0%	5	35.7%	0	.0%
Resistant	1	12.5%	1	7.1%	0	.0%
Total	8	100.0%	14	100.0%	1	100.0%

FIGURE 45: POLYMIXIN B SENSITIVITY PATTERN (n=23)



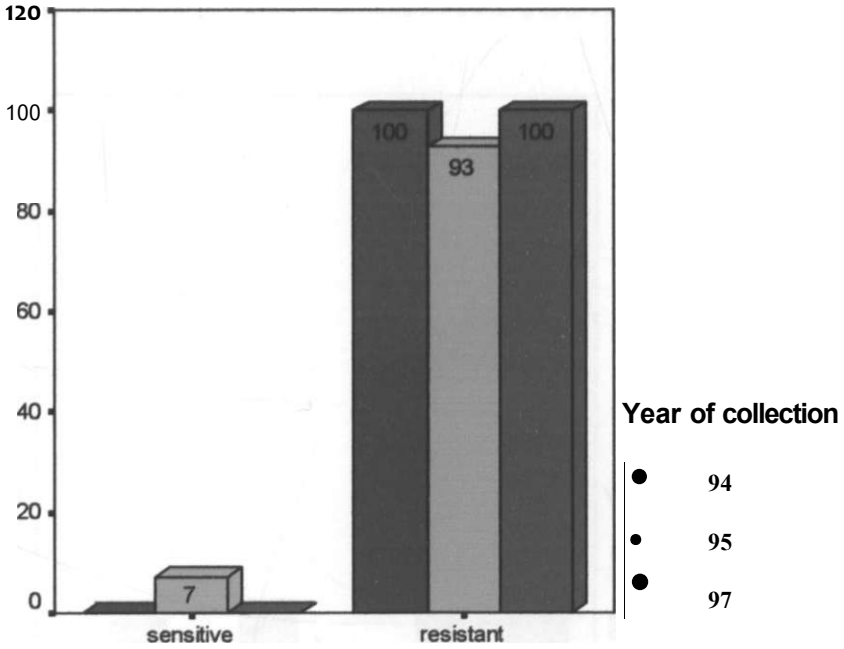
Polymixin B/S.**aureus**

S.aureus remained sensitive to Polymixin B. There had been a gradual decrease in the resistance over the years (P=0.369)

TABLE 48:CHLORAMPHENICOL SENSITIVITY PATTERN (n=26)

Tetracycline	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
Sensitive	0	.0%	1	7.1%	0	.0%
Moderate sensitivity	0	0%	0	0%	0	0%
Resistant	9	100.0%	13	92.9%	1	100.0%
Total	9	100.0%	14	100.0%	1	100.0%

FIGURE 46: TETRACYCLINE SENSITIVITY PATTERN (n=24)



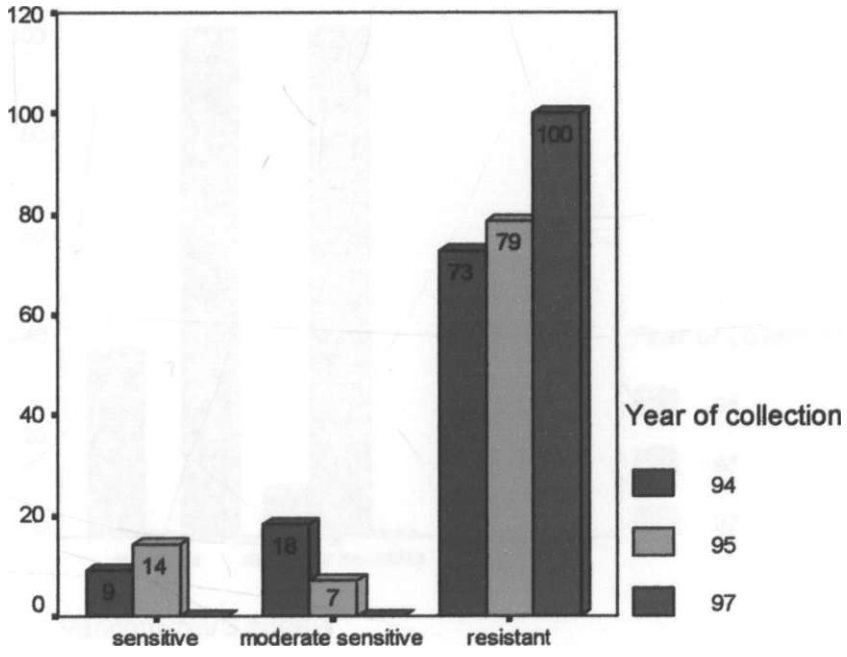
Tetracycline/S. aureus

S.aureus had remained highly resistant to Tetracycline over the years **(P=0.689)**

TABLE 49: TRIMETHOPRIM SENSITIVITY PATTERN (n=16)

Tobramycin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
Sensitive	1	9.1%	2	14.3%	0	.0%
Moderate sensitive	2	18.2%	1	7.1%	0	.0%
Resistant	8	72.7%	11	78.6%	5	100.0%
Total	11	100.0%	14	100.0%	5	100.0%

FIGURE 47: TOBRAMYCIN SENSITIVITY PATTERN (n=30)



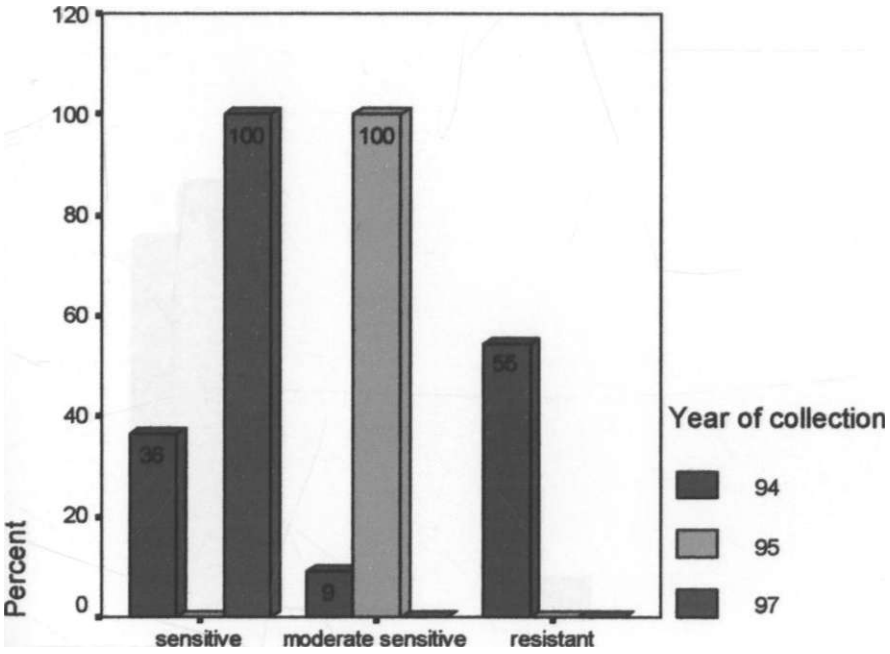
Tobramycin/S.aureus

There was a steady increase in the resistance of Tobramycin over the years (P=0.655)

TABLE 50:CEFTAZIDIME SENSITIVITY PATTERN (n=5)

Vancomycin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
Sensitive	4	36.4%	0	.0%	1	100.0%
Moderate sensitive	1	9.1%	1	100.0%	0	.0%
Resistant	6	54.5%	0	.0%	0	.0%
Total	11	100.0%	1	100.0%	1	100.0%

FIGURE 48: VANCOMYCIN SENSITIVITY PATTERN (n=13)



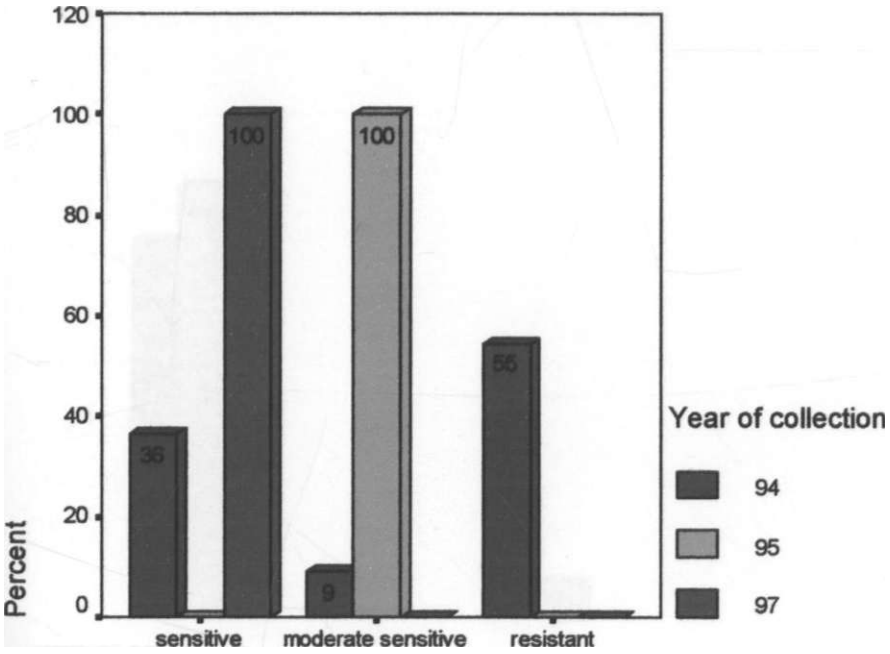
Vancomycin/S.aureus

There was a fall in the resistance of Vancomycin from 1994 to 1997 (P=0.109)

TABLE 85:CEFTAZIDIME SENSITIVITY PATTERN (n=5)

Vancomycin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
Sensitive	4	36.4%	0	.0%	1	100.0%
Moderate sensitive	1	9.1%	1	100.0%	0	.0%
Resistant	6	54.5%	0	.0%	0	.0%
Total	11	100.0%	1	100.0%	1	100.0%

FIGURE 48: VANCOMYCIN SENSITIVITY PATTERN (n=13)



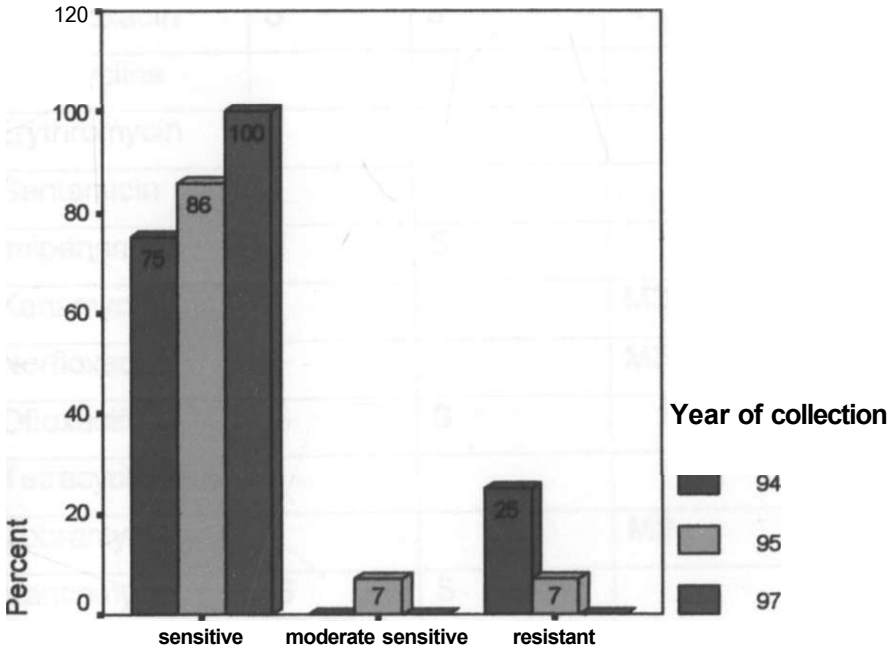
Vancomycin/S. aureus

There was a fall in the resistance of Vancomycin from 1994 to 1997 (P=0.109)

TABLE 51: OXACILLIN SENSITIVITY PATTERN (n=22)

Oxacillin	Year of collection					
	94		95		97	
	Count	%	Count	%	Count	%
Sensitive	3	75.0%	12	85.7%	4	100.0%
Moderate sensitive	0	.0%	1	7.1%	0	.0%
Resistant	1	25.0%	1	7.1%	0	.0%
Total	4	100.0%	14	100.0%	4	100.0%

FIGURE 49: OXACILLIN SENSITIVITY PATTERN (n=22)



Oxacillin/S.aureus

There was a steady increase in the sensitivity of Oxacillin over the years (P=0.689)

OVERALL SENSITIVITY PATTERN OF ANTIBIOTICS '94 - [4]97

	Sensitive Antibiotic		Moderately sensitive		Resistant Antibiotic	
	CNS	S. aureus	CNS	S. aureus	CNS	S. aureus
Amoxicillin					R	R
Ampicillin					R	R
Carbenicillin	S			MS		
Cephalexin	S	S				
Cefalotin	S	S				
Cefotaxime	S	S				
Ceftazidime			MS			
Clindamycin	S	S				
Chloramphenicol	S			MS		
Ciprofloxacin	S	S				
Doxycycline						R
Erythromycin					R	R
Gentamicin					R	R
Imipenem	S	S				
Kanamycin			MS	MS		
Norfloxacin			MS	MS		
Ofloxacin	S	S				
Tetracycline					R	R
Tobramycin			MS			R
Vancomycin	S	S				
Oxacillin	S	S				
Colistin	S					
Polymixin B	S	S				
Penicillin					R	

S = Sensitive Antibiotic
MS = Moderately Sensitive Antibiotic
R = Resistant Antibiotic

7. DISCUSSION

The data for this study was collected both at KNH and KEU from subjects with no ocular surface pathology. Both these institutions undertake a high number of eye patients and also serve as referral centres for ocular conditions.

The data collected from KEU was from patients who were going for cataract surgery and the conjunctival swabs were taken from them prior to instillation of prophylactic antibiotic drops or cleaning of the conjunctival sac with povidone-iodine.

Twenty of the total 284 questionnaires were excluded for the following reasons: Nine were on topical antibiotic medications which are known to alter the conjunctival normal flora, Three had corneal ulcers whose exact causative agent was not known. Three questionnaires had the data entered incompletely, Two volunteers were scheduled for TPR, One had buphthalmos and One had an anterior staphyloma.

From a total of Two hundred and sixty four samples collected from normal conjunctiva, One hundred and sixteen had a positive result giving a positivity of 43.94% and an odds ratio of 0.78. This is similar to results from studies in other regions of the world.[41,42,43]

Table 2 and fig. 2 show the distribution of data per the year in which it was collected. In '96 only Two people were sampled and they were culture negative, therefore these results do not appear in the analysis for the organisms isolated and the antibiotic sensitivity pattern. Most of the data collected from KEU was in '95 from preoperative cases. From previous studies[41,42,43,44,45,46,47] It has been observed that there is no difference in the distribution of either the organisms or the sensitivity pattern from the normal conjunctiva of normal people.

Fig. 3 shows the age distribution of the sample population. The youngest person is an 11 year old student and the oldest a retired 95 year old gentleman. The mean age was 46.8 yrs

In a study done in Russia on 4927 volunteers, the age range was from 22-99 yrs. The age distribution is as expected as a wide range is required to obtain a representative population since this data will be extrapolated to the Kenyan population at large.

Table 4 and fig 4 show the sex distribution of the sample population. The distribution is relatively even with a male to female ratio of 5:4

Nineteen point seven percent of the sample population were students and an equal number were reported as retired without further specification. Twenty one point six percent of the total were housewives who accounted for almost half the females. A study done on hospital workers in Brazil showed that the conjunctival flora of these workers was contaminated by the environment in which they worked. They isolated Propionibacterium, Diphtheroids, S. viridans and H. influenza. Our figures are quite low compared to other studies done. Chang and Singh isolated multiple organisms from Forty five percent of there cases[20] and Trinidade in Brazil isolated multiple organisms from Forty four point five percent of his cases though his cases were very few(18) and he managed to isolate a lot more of the transient flora.

Table 5 (fig 6) shows the percentage of organisms isolated amongst the cases. CNS was the most frequently isolated organism (41.7%),followed by S. aureus (28%). From the gram negatives Propionibacterium sp. and Corynebacterium accounted for 7.6% and 6.2% respectively. Other gram positive organisms included S. viridans, S.pneumoniae, Aeromonas sobria, S. fecalis, Pneumococcus. Amongst the gram negatives we have H. influenza, E.coli, P. aeruginosa and one isolate which was simply identified as Gram negative rods. Our figures of CNS and S. aureus compare to other studies [20,45,46,49]

Patel isolated CNS 30%, S. aureus 20%, S.peumoniae 10% amongst her controls on her study on the prevalence and bacteriology of congenital NLDO in 1999. There is a much larger isolate of S. pneumoniae in her isolates. The difference in comparison to our figures could be due to the fact that her population was predominantly children below one year of age[50].

Table 6 shows the distribution of the organisms as per the year of collection. There was no change in the distribution pattern of the organisms over time and the pattern of normal flora essentially remained the same.

7.1 ANTIBIOTIC SENSITIVITY PATTERNS

The antibiotic sensitivity was tested against Twenty seven antibiotics. The Four groups of penicillin's were tested. There were 4 Cephalosporins tested representing the 1st, 2nd and 3rd generation of the Cephalosporins. No 4th generation Cephalosporins had been included. Four Aminoglycosides were tested and they included Gentamicin, Kanamycin, Neomycin and Tobramycin. There were Three Flouroquinolones tested namely, Ciprofloxacin, Ofloxacin and Norfloxacin. Doxycycline, Erythromycin, Tetracycline and Lincomycin were the Four Macrolides tested. The other antibiotics tested were Chloramphenicol, Clindamycin, Colistin, Imipenem, Polymixin B, Trimethoprim and Vancomycin.

Table 7/Fig 8 and Table 31/Fig 31 show the sensitivity pattern of Amoxicillin a penicillin with an extended spectra of activity. Both CNS and S.aureus have a high level of resistance to this antibiotic. In previous studies done by Patel and Gichangi, both have shown a similar level of resistance. Although both Patel and Gichangi have examined pathologies and not normals, we can compare our resistance patterns since the organisms have been shown to share the same genetic pattern from other studies. The same organisms have been shown to be causative agents in some of the infections[36,50]

Table 8/Fig 9 and Table 32/Fig 32 show the sensitivity pattern of Ampicillin. Both CNS and S.aureus have a high resistance to this antibiotic. S.aureus shows an increase in the resistance from 1994 to 1997. Patel's results were comparable though the resistance of CNS (70%) and S.aureus (60%) was lower[50].

For both Amoxicillin and Ampicillin, the high and raising resistance could be related to the fact that these are the most widely and abusively prescribed systemic antibiotics in the region and the ocular normal flora do share cross resistance for antibiotics with other strains

Table 9/Fig 10 and Table 33/Fig 33 show the sensitivity pattern of Carbenicillin an antibiotic with anti pseudomonal activity. CNS had good sensitivity all through the years, whereas S.aureus is shown to have moderate sensitivity to Carbenicillin. Carbenicillin is an infrequently used antibiotic in this region and this could explain the consistently good sensitivity of the antibiotic over the years. In this study, Carbenicillin was not tested against the Pseudomonas.

Table 25 shows the sensitivity pattern of Penicillin (not specified Pen G or Pen V). Only 1 case of CNS was tested against the antibiotic in 1994 and this showed it to be resistant. There was no sensitivity pattern for S.aureus. The antibiotic had an overall high resistance with a resistance of 100% in 1997. This is the most commonly used systemic antibiotic in the region for the treatment of bacterial infections. Mundia found a resistance of 72% to Penicillin in his study at KNH & Pumwani Hosp.[51]

Table 30/Fig 30 and table 51/Fig 49 shows the sensitivity pattern of Oxacillin, a Penicillin resistant to penicillinase (in the same group as Methicillin). CNS and S.aureus both maintained a high sensitivity to Oxacillin. There is an overall increase in the sensitivity of the antibiotic from 1994 to 1997 for S. aureus increasing from Seventy five to Hundred percent in 1997, the antibiotic had remained a good antibiotic all through, however Gichangi [52] demonstrated a high resistance to Oxacillin (>50%) for most of the organisms except CNS (<50%). A change in the sensitivity pattern of Oxacillin could be indicative of a change in the sensitivity pattern of other penicillins resistant to penicillinase too. Resistant strains are usually cross resistant to the Cephalosporins, Aminoglycosides and the Macrolides[52]

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Methicillin was not tested in this series whose sensitivity pattern can give a guideline on the sensitivity of the organisms to the penicillinase resistant Penicillins. Incase of resistance then the data can be used to generalize that the bacteria especially the nosocomials are resistant to the penicillinase resistant antibiotics.[52]

From the above observations, it is evident that CNS and S.aureus the 2 most frequent micro biota of the conjunctival normal flora, had a high resistance to the Penicillins except the Penicillins with anti Pseudomonal activity (Carbenicillin) and the Penicillins with antipenicillinase activity (Oxacillin).

Table 29/Fig 29 show the sensitivity pattern of Vancomycin, a cell wall synthesis inhibitor which is highly active against Gram positive cocci and the drug of choice against MRSA (Methicillin resistant Staph, aureus)[52]. CNS was highly sensitive to Vancomycin. It had no resistance in any of the 3 years tested. S.aureus showed a decrease in resistance with a moderate sensitivity of One Hundred percent in 1995. The figures show that CNS and S.aureus remain highly sensitive to Vancomycin and its sensitivity has not changed with time. Since the normal flora consists mainly of CNS and S.aureus in our region, Vancomycin remains the first line (DOC) against conditions such as endophthalmitis for intravitreal administration.

Table 10/Fig 11 and table 34/Fig 34 show the sensitivity pattern of Cephalexin, a 1st generation cephalosporin to CNS and S.aureus respectively . The sensitivity of the antibiotic remained good (>80%) overall for both the microbiota. There is also a decrease in the resistance from 1994 to 1997 for CNS. In this study the organisms mainly isolated are the gram positive organisms and the 1st generation antibiotics are known to have good sensitivity against the gram positive organisms. In previous studies done in the region Mundia got a resistance of 5% to CNS and 40% to S.aureus[50,51]. Cephalexin has generally been a good antibiotic in the region.

Table 11/Fig 12 and table 35/Fig 35 show sensitivity pattern of Cefalotin a 2nd generation cephalosporin which are known to have activity against the gram negative organisms mainly. CNS had a high sensitivity to the antibiotic all through the years and S.aureus had moderate sensitivity. The figures are interpreted with caution due to the low figures obtained in 1997. The antibiotic was resistant to H. influenza but sensitive to P. aeruginosa. Cefalotin had good sensitivity to the Diphtheroids and Propionibacterium sp. Previous investigators in the region did not test the sensitivity to Cefalotin.[5tK 51]

Table 12/Fig 13 (CNS) and table 36/Fig 36 (S.aureus) show the sensitivity pattern of Cefotaxime. The antibiotic has low resistance to CNS, it remained a good antibiotic with high sensitivity. S.aureus shared increasing sensitivity to the Cefotaxime rising from 73% in 1994 to 100% in 1997.

Table 13/Fig 14 show the sensitivity pattern of Ceftazidime against CNS. There were 2 cases tested in 1994 with 50% resistance and 3 cases in 1995 all of which were sensitive. There was no data for 1997 and none of the S.aureus was tested against Ceftazidime

Both the antibiotics are 3rd generation Cephalosporins. Both the antibiotics have remained antibiotics with good sensitivity over the years although Cefotaxime had a slightly better sensitivity as compared with Ceftazidime. The P values for both the antibiotics were not significant for a change in the sensitivity patterns. This result could be explained by the fact that Ceftazidime is a more commonly used antibiotic than Cefotaxime in the country as a whole hence the better sensitivity of the latter.

In general the results do show us that the Cephalosporins have a good sensitivity pattern to CNS and S.aureus and this may be due to the fact that Cephalosporins are not as commonly used as other groups of antibiotics.

Table 14/Fig 15 show the sensitivity pattern of Chloramphenicol, a commonly used topical antibiotic in our region which acts by binding the 50s subunit of the bacterial ribosome and preventing protein synthesis.[52] The sensitivity of Chloramphenicol has gradually changed overall from 1994 to 1997 for both CNS and S.aureus. CNS shows a gradually increasing sensitivity to chloramphenicol. The sample size however was low in 1997 (3). S.aureus also shows a statistically significant reduction in the resistance. It is moderately sensitive to chloramphenicol. In the study done by Mundia [50], the sensitivity was 13% and Patel [51] got a sensitivity of 25% to Chloramphenicol. Our figures for 1994 and 1995 are comparable, although the figures for 1997 do show a significant fall in the resistance.

This could be explained by the fact that Gentamicin was a more commonly used antibiotic and a 1st line, hence Chloramphenicol having retained a good sensitivity. Secondly Chloramphenicol is used more in the pediatric group against suspicion of H.influenza and in adults in meningitis. This may explain good sensitivity of the antibiotic during that period

Table 16/Fig 17 show the sensitivity pattern of Clindamycin, an antibiotic with similar action to Chloramphenicol. Its main uses are in the treatment of gram positive and anaerobic gram negative organisms.[52] CNS had remained highly sensitive to Clindamycin from 1994 to 1997. Mundia got a resistance of 32% in his study[51], whereas Gichangi showed the antibiotic to be highly sensitive which is comparable to this study[36] therefore Clindamycin has remained a good antibiotic all through (>80% sensitivity). S.aureus shows a raising sensitivity to Clindamycin from 1994 to 1997. Clindamycin is not a commonly prescribed antibiotic in our region and this may be the reason for the good sensitivity demonstrated by both the microbiota.

The three aminoglycosides studied included Gentamicin, Tobramycin and Kanamycin. In Table 19/Fig 20, CNS show a statistically significant increase in its resistance to Gentamicin from 1994 to 1997. Table 42/fig 41 shows that S.aureus has increasing resistance to Gentamicin with an overall high resistance, CNS shows a reduction in the sensitivity of Gentamicin from 1994 to 1995 whereas S.aureus shows a mild increase in its sensitivity to Kanamycin. There were no sensitivity tests done for Kanamycin in 1997, but the results for

1994 and 1995 do show a raise in the resistance to the antibiotic. CNS shows a reducing sensitivity to Tobramycin and S.aureus shows it has remained highly resistant to Tobramycin with an overall raise in its resistance. The overall raise in resistance could be explained by the fact that there may be cross resistance to the antibiotics considering the fact that Gentamicin is a very frequently used antibiotic in our setting. The mechanism of cross resistance may most likely be enzymatic modification of the drug which is both inactivated and prevents uptake of the active drug. [52]

Out of the 11 Streptococcus sp. isolated, 7 were tested for Gentamicin and 6 tested resistant (85%), 5 were tested for Neomycin and 4 tested resistant whereas 5 (80%) were tested for Tobramycin and 4 tested resistant (80%). Although the results show that Streptococci sp. are highly resistant to the Aminoglycosides, it has been demonstrated that they require such high MIC's, that they are often found to be resistant to the Aminoglycosides using the conventional susceptibility testing.[52]

There were two Tetracyclines studied in this series, Doxycycline and Tetracycline. These antibiotic acts by blocking the 30s subunit of the bacterial ribosome and prevent attachment of the transfer RNA hence preventing protein synthesis. There was 1 sample of S.aureus tested against Doxycycline and this was resistant to the antibiotic. There were no results for CNS tested. CNS (table 26/fig 26) showed a raising resistance to Tetracycline from 75% to 80% to 100% from 1994 through to 1997. This raise in the resistance was statistically insignificant, considering it was already a highly resistant antibiotic. S.aureus had been highly resistant to Tetracycline over the years with the resistance going to 100%. In previous studies done[36,50,51], all have shown a high degree of resistance to the Tetracyclines and their figures are comparable to our figures. This high resistance could be attributed to the wide and injudicious use of the Tetracyclines in our setup, including use as lubricant when no other appropriate ophthalmic lubricant is available.

Table 18/Fig 19 shows the sensitivity pattern of Erythromycin, a macrolide antibiotic which acts by blocking the 50s subunit of the bacterial ribosome hence preventing protein synthesis[52]. CNS shows a mild increase in the resistance to the antibiotic from 1994 to 1997. Mundia showed a resistance of 32%, Patel had a resistance of 30% and Gichangi showed CNS to be highly resistant. S.aureus shows a reduction in its sensitivity to the antibiotic with a corresponding raise in the moderate sensitivity and the resistance to Erythromycin. In other studies done[36,50,51],Mundia showed resistance of 20%, Patel showed resistance of 30% and Gichangi showed a resistance of 50% their figures compare well with this study indicating that the antibiotic does have a relatively higher resistance. However the figures of Gichangi are comparatively higher. The higher resistance can be attributed to the wide and injudicious use of the antibiotic especially the ointment form in our setup for the treatment of various eye conditions and infections. However its resistance is much lower in comparison to the Tetracyclines.

The 1st and 2nd generation Flouroquinolones studied included Ciprofloxacin, Norfloxacin and Ofloxacin. Table 15/Fig 16 show the sensitivity pattern of CNS to Ciprofloxacin. The sensitivity has remained good overall though there is a mild increase in the resistance in 1997 (3 samples). CNS has good sensitivity to Norfloxacin from 1994 to 1997. Due to the low number of cases in 1997, the pattern cannot be interpreted reliably. CNS has remained highly sensitive to Ofloxacin all thorough the years. This was the first topical Flouroquinolone to be introduced in the market. Table 39/rig38 show the sensitivity of S.aureus to Ciprofloxacin. There is no statistically significant change in the sensitivity pattern. The antibiotic has remained highly effective all through. Table 45/Fig 44 show S.aureus has remained sensitive to Norfloxacin and Table 46 shows S.aureus has remained highly sensitive to Ofloxacin. The emerging resistance to Ciprofloxacin and Norfloxacin could be due to the increasing systemic and local usage of the two Flouroquinolones and the ready availability of the antibiotics in the market. Ofloxacin was not widely used in 1994 and 1997. Our figures are similar especially for Ciprofloxacin with that of Patel (20%)[50], but higher then that of Gichangi (no resistance)[36] and Mundia (no resistance)[51]. However our figures for Norfloxacin and Ofloxacin are comparable with these studies. Other studies have shown that there is raising emergence of resistance to the Flouroquinolones especially Ciprofloxacin. Our results follow suit for

Ciprofloxacin and Norfloxacin but Ofloxacin had remained an antibiotics with low resistance.[52,54]

Table 17/Fig 18 show the sensitivity pattern of CNS to Colistin. The sensitivity had significantly increased from 1994 to 1995, Other studies done in the region have not included the sensitivity pattern of Colistin. This antibiotic was not used as a systemic antibiotic in our region.

Table 20/Fig 21 show the sensitivity pattern of Imipenem, a Carbapenem whose structure is similar to the Penicillins. This antibiotic has remained highly sensitive against CNS and S.aureus. This antibiotic had not been tested by other researchers in the region. Since the antibiotic was not being used widely in our region at that time, this could explain the maintenance of the good sensitivity all through from 1994 to 1997

Table 24/Fig 25 and Table 47/Fig 45 show the sensitivity pattern of Polymixin B a surfactant which interacts with the cell membrane of bacteria disrupting the osmotic integrity of the membrane. The antibiotic was studied in 1994 and 1995. The resistance of CNS and S.aureus has remained low all through This is not a commonly used antibiotic except in combinations with other topical antibiotics or steroids. This antibiotic is not utilised as a systemic antibiotic, and this could possibly explain the low overall resistance

Table 29/Fig 30 show the sensitivity pattern of Trimethoprim, a Sulfonamide which were the 1st group of chemotherapeutic agents used in the prevention and the treatment of bacterial infections in humans. The antibiotic inhibits the bacterial synthesis of folic acid. This study showed a significant reduction (63%-0%) in the resistance of the antibiotic from 1994 to 1997. This could be attributed to the decrease in use of the antibiotic in place of alternative antibiotics and due to the fact that there is no topical preparation of the antibiotic available in the country. In other studies it has been seen that, when Trimethoprim is used in combination with other antibiotics, it has similar efficacy to other antibiotics.[55]

The above results are laboratory results where the Kirby disc diffusion technique was utilized to test the sensitivity pattern of the antibiotics. The concentrations tested on these discs tests for the blood concentrations achieved by the standard systemic dosages of these antibiotics. To the contrary, the concentrations achieved by the local applications of these antibiotics to the eye are much higher therefore these results give an orientation but the clinical response is decisive.

8. CONCLUSION

a. Penicillins have been shown to have high resistance. Amoxicillin and Ampicillin should not be used as first line in the treatment of bacterial eye infections in our region

b. Cephalosporins have been shown to be good antibiotics with a good sensitivity pattern. They should be used as first line treatment in bacterial eye infections such as preseptal cellulitis, bacterial conjunctivitis

c. Vancomycin remains the DOC for intravitreal injections since it maintains a very good sensitivity pattern

d. Ceftazidime is not the best alternative for intravitreal use or in the treatment of severe bacterial eye infections since its sensitivity does not compare well with the other third generation cephalosporins.

e. There is increasing resistance to Aminoglycosides especially Gentamicin in the conjunctival flora:
   a) avoid in very acute infections
   b) the clinical picture decides in other conditions (refer to the concentration discussion) but increasing signs indicate failure

f. Commonly used antibiotics all have a high resistance or an increasing resistance such as Tetracycline, Gentamicin, Amoxicillin, Ampicillin, Penicillin

9. RECOMMENDATIONS

1. There is a need for continued testing of antibiotics for changing sensitivity pattern locally, since this will influence our prescribing habits. The study shows that antibiotics prescribed commonly in our region has either a high resistance or an emerging resistance

2. There is a need for testing of Ceftriaxone and Metronidazole (commonly used antibiotic for covering anaerobic organisms) in our region since these antibiotics are commonly prescribed and no sensitivity pattern has been established

3. Ceftazidime has been shown to have some resistance therefore an alternative such as cefotaxime which has low resistance should be utilized for intravitreal injections

4. Conventional methods should not be used to test especially Streptococci for sensitivity against aminoglycosides due to the high MIC required. Instead alternative methods should be utilized such as the tube dilution method

5. Tetracycline should be used cautiously for treatment of infections in the eye due to its high resistance. Instead it should be utilized for its other properties in the treatment of non infectious eye conditions

6. There is a need for a study on 3rd and 4th generation Flouroquinolones since they are increasingly being used both as topical and systemic antibiotics

7. There is a need for a study on the sensitivity pattern of Azithromycin since its going to be utilized as an alternative in the treatment of trachoma

There is a need for a study on the conjunctival flora of children and their antibiotic sensitivity pattern

Avoid indiscriminate use of antibiotics.

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APPENDIX 1

COLLECTION AND PROCESSING OF SPECIMEN

1. PROCESSING

The culturing of positive results depends on the use of appropriate methods of cultivation [5]

The specimen received in the laboratory was verified for patient name, patient number and then immediately processed.

Smears were made from the specimen and stained appropriately which were then subjected to direct microscopic examination.

After 24hrs of incubation at 37°C (28°C for fungal cultures), the plates were inspected for colony growth which were then processed for identification. In case of no growth the plates are reincubated for further 24hrs before reporting it as a negative growth.

2. COLLECTION

Conjunctival specimen was collected using a conjunctival swab which was a moist cotton sterile swab stick which was transported in a BHI broth. The collected specimen was taken and processed in the laboratory within half an hour of collection.

3. STAINING OF SPECIMEN

3.1 POTASSIUM HYDROXIDE (10% KOH) WET MOUNT

MATERIALS

1. KOH	lOg
2. Glycerin	lOmls
3. Distilled water	80mls

PROCEDURE

1. The above materials are mixed together and stored at room temperature.

2. The materials to be examined are placed on a glass slide and a drop of 10% KOH is added.

3. The material is covered with a cover slip and allowed to remain at room temperature for approximately 10 min.

4. Examination under dry power objective for hyphal elements, budding cells and other fungal morphologies is conducted

3.2 GRAM'S STAINING TECHNIQUE

PROCEDURE

1. A thin smear either from the culture or from the specimen was placed on a glass slide
2. The smear was then heat fixed
3. The heat fixed smear was then flooded with crystal violet for lmin
4. This was washed off with distilled water
5. The slide was then flooded with Gram's iodine for lmin
6. Iodine was then washed with distilled water
7. Decolorize rapidly with acetone: alcohol (1:1) for 10 seconds
8. The slide is then washed with distilled water
9. The slide is then flooded for lmin with dilute carbol fiischin, which is then washed with distilled water
10. Finally the slide is air dried

Under the oil immersion objective the Gram positive bacteria appear dark purple and the Gram negative appear pink. Yeast like fungi which appear as gram positive budding yeast cells can also be demonstrated.

APPENDIX II (QUESTIONNAIRE : Normal flora)

Date Patients name_____No._____Birth date

Occupation Physician Sex Residence

HISTORY

H/o Discharge Y N
Medication usage Steroids Y N
Antibiotics Y N
Others : specify
Systemic review DM Asthma Allergy Rheumatism CV insufficiency
(Insert Y/N) Renal insufficiency

OCULAR SURFACE EXAMINATION

Conjunctiva normal abnormality: specify
Lids normal abnormality: specify
Cornea normal abnormality

LABORATORY PROTOCOL

MEDIA	D1	D2	D3	D4	D5	D6	D7
BA							
CA							
THIO							

ORGANISM/S
ISOLATED

ANTIBIOTICS	SENSITIVTTY(mm)			ANTIBIOTICS	SENSI1**riVITY**(mm)		
	S	MS	R		S	MS	R
Amoxicillin				lmipenem			
Ampicillin				Kanamycin			
Carbenicillin				Lincomycin			
Cefalexin				Neomycin			
Cefalotin				Norfloxacin			
Cefotaxime				Ofloxacin			
Ceftazidime				Polymyxin B			
Chloramfenicol				Penicillin			
Ciprofloxacin				Rifampicin			
Clindamycin				Tetracycline			
Colistin				Trimethropin			
Doxycycline				Tobramycin			
Erythromycin				Vancomycin			
Gentamicin				Oxacillin			

APPENDIX III

CONSENT

I hereby agree to undergo the procedure of swabbing of my conjunctiva for the purposes of a study on the "Conjunctival normal flora among Kenyans". It has been explained to me by Dr._____that the procedure will not be of any harm to me, neither will it have any side effects on me. In the event of any untoward effect due to the procedure being conducted, all the appropriate care will be administered to me.

The data collected from this procedure will be treated in the strictest confidence and no names will be mentioned anywhere during the data analysis or in the discussion. It has been explained to me that no information will be disclosed to any persons not party to the study. It has been explained to me that my participation in this study is voluntary and I may withdraw at any point during the study without victimization

I am allowed to ask the data collector any questions in relation to the procedure being conducted.

Signed_____participant

Signed_____data collector

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