



Etiology of Ocular Infections and Minimum Inhibitory Concentration of Multidrug- Resistant *Staphylococcus aureus* Isolates to Vancomycin, Ciprofloxacin and Chloramphenicol

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Abstract

Introduction: Ocular infections constitute a significant portion of curable eye diseases. However, in developing countries like Nepal, bacterial and fungal spectrum is not well established for ocular infections. Therefore, ocular infections still remain as the major cause of blindness. This study was sought to determine the bacterial and fungal etiology of various ocular infections and assess the antibiotic susceptibility pattern of the bacterial pathogens.

Methods: This cross-sectional study was conducted at BP Koirala Lions Center for Ophthalmic Studies, Kathmandu, Nepal from June 2012 through April 2013. Various ophthalmic specimens of clinical values such as the corneal scrap, conjunctival swab and eye pus and biopsy materials were collected aseptically and processed for culture. After sufficient incubation, isolates were identified by colony morphology, Gram staining and relevant biochemical tests. Identified bacteria; isolates were then subjected under Antibiotic Susceptibility test (AST) by modified Kirby-Bauer disc diffusion method to determine Multi Drug Resistant (MDR) isolates and Minimum Inhibitory Concentration (MIC).

Results: A total number of 810 ocular specimens, mainly from the case of conjunctivitis (644/810) were processed. Of which, 31.97% (270/810) showed growth on culture medium: 267 (98.85%) were bacterial, 2 (0.77%) were fungal (*Penicillium* spp and *Fusarium* spp) and 1 (0.38%) was yeast (*Rhodotorula*) isolate. Gram positives alone accounted for 96.25% (257/267) among bacterial isolates. *Staphylococcus aureus* (52.96%) and *Escherichia coli* (1.48%) were the most common Gram positive and Gram-negative isolates respectively. Gram positive isolates showed resistance to ciprofloxacin (39.29%), cephalexin (38.13%) and amikacin (31.52%) while gram negatives were resistant to cefazolin (80%). All isolates of *E. coli*, *Hemophilus influenzae* and *Serratia* spp together with 48.95% of *S. aureus* were MDR. Among 5 methicillin resistant *S. aureus* (MRSA) isolates, 2 isolates were vancomycin intermediate *S. aureus* (VISA). Further, the minimum inhibitory concentration (MIC) break points for *S. aureus* to chloramphenicol, ciprofloxacin and vancomycin were also determined.

Conclusion: Conjunctivitis among adults was the most prevalent case found so far, yielding higher culture positivity. Along with MDR bacterial agents, fungal agents were observed to be posing significant threat in the disease management. Early diagnosis, proper management and empirical treatment are advised to prevent infections related blindness.

Keywords: Ocular Infections; Conjunctivitis; AST; MDR; MRSA; VISA; MIC

Introduction

There are various ocular and periocular microbial infections including cellulitis, blepharitis, chalazion, conjunctivitis, keratitis, uveitis, endophthalmitis, panophthalmitis, episcleritis, scleritis, pterygium, dacryocystitis, internal and external hordeolum, canaliculitis [1]. Ocular redness, swelling, tearing or discharge,

photophobia, ocular pain or discomfort, or foreign body sensation are most common symptoms observed during ocular infections [2]. *S. aureus*, *S. pneumoniae*, *Bacillus cereus*, Coagulase-negative *Staphylococci* (CONS), *Neisseria gonorrhoeae*, *N. meningitidis*, *Hemophilus influenzae*, *Moraxella catarrhalis*, *Pseudomonas* spp, Enteric Gram-negative bacilli, *Corynebacterium* spp, alpha-haemolytic

Streptococci, *Micrococcus* spp, Beta-haemolytic *Streptococci*, *Corynebacterium diphtheriae* are the common bacterial agents for eye infections like conjunctivitis, keratitis and endophthalmitis [1,3]. Fungal infections are caused as opportunistic infections. *Fusarium* spp, *Aspergillus* spp, *Penicillium* spp, *Scedosporium* spp, *Paecilomyces* spp, *Acremonium* spp, *Bipolaris* spp and yeasts like *Candida* spp, *Cryptococcus* spp, *Rhodotorula* spp, are the major agents to bring the infection [4].

Various socio-demographic factors like age, marital status, income also determine the rate of infection [5]. Globally about 202 million people are visually impaired. The global prevalence of blindness is 0.7% ranging from 0.3% in the developed countries to 1.4% in sub-Saharan Africa [6].

Corneal scarring, tradition remedies, civil war, insufficient medical care, and Vitamin A deficiency (VAD) among children and pregnant women remain as the leading causes of blindness in Nepal. Some eye infections are the result of occupational hazard basically observed among agricultural workers of India and Nepal, often caused by organic matter such as rice paddy stalks, dust, grass, wood and animal products [7]. Dirty water, mud and even cow dung used as part of traditional remedies further worsen the case which could otherwise be treated up to 80% of the cases, if diagnosed promptly [8].

The etiology of ocular infections varies considerably between and within countries [9]. Due to geographical diversity with a known climatic variation, several types of diseases more frequently occur in Nepal [10]. While the poor health facilities, illiteracy, belief on traditional treatments delaying the clinical diagnosis and treatments are the major causes of blindness [11]. Wide range of organisms produce a similar clinical picture, as a result clinical diagnosis does not give an unequivocal indication of the causative organisms [12,13]. The initial antimicrobial therapy is often guided by subjective interpretation of clinical features presented [14].

Ocular pathogens are developing resistance towards antibiotics in higher rates that may have serious consequences such as keratitis, endophthalmitis, orbital cellulitis, or panophthalmitis [15]. Nowadays resistance of bacteria to antimicrobial agents has become a worldwide concern. In a study, significant portion of *S. pneumoniae* and *S. aureus* had shown resistance to macrolide antibiotic agents, penicillin, and older fluoroquinolones. However, new generation of fluoroquinolones including third generation levofloxacin and fourth generation moxifloxacin and gatifloxacin have been introduced to counter this problem [16]. In infections

like conjunctivitis and corneal infection, topical drops are preferred while Intravitreal antibiotics and possibly subconjunctival and parenteral antibiotics are preferred for endophthalmitis and infection of deep adnexal structures [17]. Among 85% of methicillin-resistant *S. aureus* (MRSA) isolates were resistant to topical fluoroquinolones first-line treatment of ocular infections-- includes the newer 8-methoxy fluoroquinolones, gatifloxacin and moxifloxacin [15]. Similarly, some strains of *H. influenzae* were resistant to chloramphenicol and ampicillin [18].

Emergence of antibiotic resistant strains like MRSA, methicillin resistant *S. epidermidis* (MRSE), vancomycin resistant *Staphylococcus aureus* (VRSA) and multidrug resistant (MDR) strains of *Pseudomonas* spp, *Haemophilus* spp, *Streptococcus* spp are the major problem in hospital environment. They are associated with increased duration of hospital stay, disseminated infection, increased socioeconomic cost and furthering adverse complications in untreated cases. The study was conducted aiming to identify etiological agent of specific ocular infections and to know the changing resistance pattern of those isolates. Further, the study was conducted to determine the prevalence of MRSA and to evaluate the MIC values of *S. aureus* to vancomycin, ciprofloxacin and chloramphenicol. The study thus helps in proper diagnosis and empirical therapy of ocular infections in all Hospital settings.

Materials and Methods

Study period, site and population

This hospital-based cross-sectional study was carried out at B.P. Koirala Lions Centre for Ophthalmic Studies (BPKLCOS), Institute of Medicine, Kathmandu, Nepal from June 2012 through April 2013. Study was conducted within the population defined by physician as the probable case of ocular infections (both bacterial and fungal eye infections) and showing specific clinical symptoms and visiting the respected tertiary eye care center. Patients undergoing ocular surgery and patients on long term treatment for systemic diseases (on steroids, immunosuppressive or anti-cancer drugs) were not included in the study.

A total of 810 different ocular samples were collected from the patients suspected of ocular infections. The personal and demographic information such as personal details, clinical history, clinical symptoms, were obtained by using requisition file of hospital and a structured questionnaire. The laboratory analysis of collected samples was done in pathology laboratory of BPKLCOS and KCMS. All the ocular samples transferred to the laboratory were processed during the study period.

Laboratory processing

Sample collection

Eye specimens such as eye swabs, corneal scrapings, aspirate of vitreous and aqueous fluids, corneal button, were collected by trained physician [19]. The conjunctival surface samples were taken with sterile cotton or calcium alginate cotton swab. For the corneal samples, a sterile Kimura spatula was used and additionally tweezers for sampling tissue remains in the compromised area. Intraocular liquid was sampled with vitreal puncture (vitreous humor) or with paracentesis (aqueous humor), in a syringe under aseptic conditions and sent to the laboratory analysis immediately.

Sample storage and transportation

The samples collected were immediately inoculated into culture media and also smears were prepared on slides for direct microscopy. Since, the quantity of eye specimen like aqueous (AC) and Vitreous (VIT) were scanty, they were processed for culture immediately. In case of delay, all the specimens were stored at 4°C until further lab analysis [20].

Microscopic examination

The smears prepared on the bedside were examined by following standard Gram's stain technique for bacteria [21] and adhesive tape technique using lactophenol cotton blue (LPCB) and 10% potassium hydroxide (KOH) analysis for the fungi identification and then observed under microscope [20].

Culture of specimen

The samples obtained were inoculated into primary isolation media; Blood agar (BA) and Chocolate agar (CA) for isolation of bacteria and into Sabouraud's Dextrose Agar (SDA) plate for isolation of fungi. The chocolate agar plate was always inoculated first. For conjunctival swabs, the swabs of both eyes were inoculated on single media plate using the opposite half of the plate(s). In case of corneal samples, the samples were inoculated on CA and BA plates by C streaking. Whereas for Vitreous (VIT) and Aqueous chamber (AC) culture, the AC or VIT drop were directly inoculated on CA and BA plates by C-streaking. A C-shaped streak was made on culture plates to ensure that growth was from the specimen and not laboratory contaminants. Thioglycolate broth media was used to enrich the both aerobic and anaerobic microorganisms. In case of scant amount of specimen of intraocular fluid submitted in syringe, broth was used to wash out the syringe by drawing up small amount of broth.

The cultures on BA and CA were incubated at 37°C in 5 - 7% CO₂ for 48 hrs. The broth media were observed for the turbidity. The

cultures were checked at 24 and 48, reporting the microbial growth in each culture. In the absence of growth, the bacterial culture media were discarded at day 5.

Similarly, the specimens inoculated for fungal culture on SDA plates were incubated at 25 - 30°C. The cultures were checked for fungal growth after 48 hours upto 4 weeks (minimum of 1 week) [20,21].

Isolation and identification of bacteria

After 24 hours of incubation, the identification procedure was followed. The standard microbiological technique which involved colony morphology, staining reaction, biochemical properties were followed for the identification of organisms [19,21].

Isolation and identification of fungi

After one week of incubation, the colony characteristics were studied and identification was done by following Standard mycological procedures [22]. Fungal hyphae and spores were observed microscopically by using adhesive tape technique [23]. Indigenous and rare organisms, if present, were reported as "Possible Contamination".

Antimicrobial susceptibility test

The antimicrobial sensitivity tests of the identified bacteria were performed on commercially available Muller Hinton Agar (MHA) of Hi-Media Company of India. The sensitivity test for *S. pneumoniae* and viridians was performed on MHA with 5% blood. Bacterial susceptibility test to antimicrobial agent was performed *in vitro* by modified Kirby-Bauer disc diffusion method using fresh broth culture of the isolates. The antibiotics used in the study included amikacin, chloramphenicol, ciprofloxacin, ceftriaxone, ceftazidime, gentamycin, vancomycin, ofloxacin, cefazoline, and the results were interpreted as sensitive, intermediate or resistant by comparing the zone of inhibition as per the guidelines of Clinical and Laboratory Standard Institute [24].

Screening of MRSA

Further screening of MRSA was conducted among multidrug resistant *Staphylococcus aureus*. *Staphylococcus aureus* strains which showed resistant to oxacillin (1 µg) at 35°C were subjected to test cefoxitin (30 µg) to confirm MRSA. In this study if isolates were resistant to at least three classes of antimicrobial agents, then it was regarded as MDR [25,26]. MRSA isolates along with other MDR isolates in pure culture were preserved in 20% glycerol containing tryptic soya broth at -70°C until further investigation.

Determination of minimum inhibitory concentration (MIC)

MIC to vancomycin in isolated MRSA and MIC to chloramphenicol and ciprofloxacin in MDR *Staphylococcus aureus* (ciprofloxacin and chloramphenicol resistant *S. aureus*) was performed by Agar dilution method following CLSI guidelines [24].

Quality control

Each antibiotics disc and Mueller Hinton agar were checked for each lot number, manufacture date and expiry date and tested for their performance quality with *S. aureus* ATCC 29213 and *E. coli* ATCC 25922. Quality of sensitivity tests was maintained by maintaining the thickness of Mueller-Hinton agar at 4 mm and the pH at 7.2 - 7.4.

Data management and analysis

The data were filled in data entry sheet and were analyzed using Statistical Package for Social Science (SPSS) software (Version 16.0) and Microsoft EXCEL.

Results

A total of 810 ocular samples were processed for culture and all culture positive samples were further processed for antibiotic susceptibility test.

Five different types of samples were collected from the suspected patients with more than twenty different infections. Highest number of samples were from the clinical cases of conjunctivitis; the sample being conjunctival swabs (n = 767; 94.69%). Here, the p value (p < 0.05) showed significant relationship between type of sample and culture positivity (Table 1).

Age and sex wise distribution for isolated organisms

Among the different age group of patients, highest number of infections were found in age group 19 - 60 years that included 325 (40.12%); male patient: 169 and female patient: 156. The relationship between age and gender with culture positivity was observed to be insignificant (P > 0.05) (Table 2).

Culture positivity according to the clinical diagnosis of infections

More than 20 different types of clinical ocular cases were included in this study. The case of acute bacterial conjunctivitis was the highest having 79.5% (n = 644) though its culture positivity was only 30.12%. The 100% culture positivity was observed in case of Impetigo and Blepharospasm Conjunctivitis though the cases reported on this disease were only 1 and 2 respectively. These cases of culture positive were followed by dacryocystitis 75% (n = 6) (Table 3).

Generalized pattern of organisms isolated from the samples

Out of the 270 different organisms isolated maximum number of organisms belong to Gram positive bacteria i.e. 239 (88.52%) (Figure 1).

Bacterial and fungal isolates identified from the samples of ocular infections

Among gram positive isolates *S. aureus* was the dominant organisms isolated; 137 out of 267 (51.31%) where as in case of Gram-negative isolates *E. coli* was frequently isolated; 4 out of 267 (1.48%). Among fungal isolates only 2 genus, *Penicillium* spp and *Fusarium* spp and only one genus of yeast *Rhodotorula* spp was isolated (Table 4).

Antibiotic susceptibility pattern observed in Streptococci isolates

Among Gram positive isolates from the ocular infections *S. pneumoniae* was found to be highly resistant to Amikacin (72.73%). Group D Streptococci were found to be highly resistant to Tobramycin (43.75%), viridans streptococci were highly resistant to Amikacin and Tobramycin (50%) and *Streptococcus pyogenes* were highly resistant to Tobramycin, Gentamycin, Chloramphenicol and Cephalexin (33.33%). *Bacillus* spp, *Micrococcus* spp and *Corynebacterium* spp were found to be susceptible to all the antibiotics used (Table 5).

Sample Type	Total Number	Culture positive for bacteria (%)	Culture positive for fungi (%)	Culture positive for yeast (%)	Total culture positive (%)	P value
Conjunctival swab	767	235 (30.64)	0 (0.00)	0 (0.00)	235 (30.64)	0.003
Corneal scraps	33	15 (45.45)	2 (6.06)	1 (3.03)	18 (54.55)	
Vitreous and Aqueous Taps	8	4 (50)	0 (0.00)	0 (0.00)	4 (50)	
Eye Pus	2	2 (100)	0 (0.00)	0 (0.00)	2 (100)	
Total	810	256 (31.60)	2 (0.25)	1 (0.12)	259 (31.98)	

Table 1: Distribution of sample collected according to its type.

Age group (year)	Male	Culture Positives (%)	Female	Culture positives (%)	Total Sample	Total Culture Positive (%)	p-value
< 1	89	42 (47.19)	35	21 (60.00)	124	63 (50.81)	
1 - 5	92	28 (30.43)	48	16 (33.33)	140	44 (31.43)	
6 - 18	100	30 (30.00)	72	18 (25.00)	172	48 (27.91)	0.354
19 - 60	169	43 (25.44)	156	41 (26.28)	325	84 (25.85)	
> 60	28	11 (39.29)	21	9 (42.86)	49	20 (40.82)	
Total	478	154 (32.22)	332	105 (31.62)	810	259 (31.98)	

Table 2: Age and sex wise distribution of patients according to culture positivity.

Note: < 1 = Infants and neonates, 1 - 5 = Toddlers, 6 - 18 = Children, 19 - 60 = Adults and > 60 = Seniors.

Clinical Diagnosis	Total number	Culture Positive for Bacteria	Culture Positive for Fungi	Culture Positive for yeast	Total Culture Positive (%)
Acute Bacterial conjunctivitis	644	194	0	0	194 (30.12)
Endophthalmitis	9	4	0	0	4 (44.44)
Corneal ulcer	21	11	2	0	13 (61.90)
Blepharoconjunctivitis	14	3	0	0	3 (21.42)
CNLDO	35	11	0	0	11 (31.43)
Pseudomembranous acute bacterial conjunctivitis	16	4	0	0	4 (25)
Preseptal cellulitis	8	3	0	0	3 (37.50)
Chalazion	4	0	0	0	0 (0)
Viral keratoconjunctivitis	8	3	0	0	3 (37.5)
Blepharospasm Conjunctivitis	2	2	0	0	2 (100)
Follicular conjunctivitis	5	0	0	0	0 (0)
Phlyctenular conjunctivitis	6	0	0	0	0 (0)
Dacryocystitis	8	6	0	0	6 (75)
Stye	8	4	0	0	4 (50)
Keratitis	5	1	0	1	2 (40)
Hordeolum	3	2	0	0	2 (66.67)
Impetigo	1	1	0	0	1 (100)
Trauma	6	3	0	0	3 (50)
Others	7	4	0	0	4 (57.14)
Total	810	256	2	1	259 (31.98)

Table 3: Culture positivity according to the clinical diagnosis of infections.

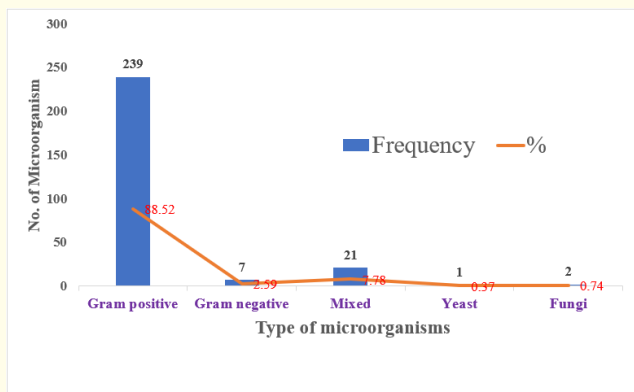


Figure 1: Growth pattern of microorganism from suspected eye infection patients.

Antibiotic susceptibility pattern observed in *Staphylococci* and *Enterococci* isolates

S. aureus is among the highly isolated Gram-positive bacteria 52.96% of total bacterial isolates. They were found to be highly resistant to ciprofloxacin (52.45%). Among 143 *S. aureus*, 3.4% (5/143) of them were found to be MRSA. Eighty percent of these MRSA isolates were found to be resistant to all cephalosporins namely: cefazolin, cephalexin and cefotaxime, amoxicillin and ciprofloxacin. Imipenem and azithromycin were found to be 100% effective on MRSA isolates. CONS were found to be 48% resistant to cephalexin while *Enterococcus* spp were found to be 57.14% resistant to ciprofloxacin and cephalexin (Table 6).

Isolates	Pure Isolates	Mixed with other bacteria	Total number (%)
Gram Positive			
<i>Staphylococcus aureus</i>	137	6	143 (52.96)
<i>Streptococcus pneumoniae</i>	52	3	55 (20.37)
CONS	23	2	25 (9.26)
Group D <i>Streptococci</i>	13	3	16 (5.93)
<i>Enterococcus</i> spp	6	1	7 (2.59)
Viridan <i>Streptococci</i>	3	1	4 (1.48)
<i>Streptococcus pyogene</i>	3	-	3 (1.11)
<i>Bacillus</i> spp	1	1	2 (0.74)
<i>Corynebacterium diphtheriae</i>	1	-	1 (0.37)
<i>Micrococcus</i> spp	-	1	1 (0.37)
Gram Negative			
<i>Escherichia coli</i>	2	2	4 (1.48)
<i>Pseudomonas</i> spp	3	-	3 (1.11)
<i>Haemophilus influenzae</i>	1	1	2 (0.74)
<i>Serratia</i> spp	1	-	1 (0.37)
Fungi			
<i>Fusarium</i> spp	1	-	1 (0.37)
<i>Penicillium</i> spp	1	-	1 (0.37)
Yeast			
<i>Rhodotorula</i> spp	1	-	1 (0.37)
Total	249	21	270 (100)

Table 4: Bacterial and fungal isolates identified from the samples of ocular infections.

Note: CONS: Coagulase Negative Staphylococci.

Anti-biotics used	<i>Streptococcus pneumoniae</i>	Group D <i>Streptococci</i>	Viridan <i>Streptococci</i>	<i>Streptococcus pyogenes</i>
	n = 55	n = 16	n = 4	n = 3
	R (%)	R (%)	R (%)	R (%)
AK	40 (72.73)	5 (31.25)	2 (50)	0 (0.00)
TB	32 (58.18)	7 (43.75)	2 (50)	1 (33.33)
G	8 (14.55)	3 (18.75)	1 (25)	1 (33.33)
C	2 (3.64)	2 (12.5)	0 (0.00)	0 (0.00)
CIP	7 (12.73)	6 (37.5)	0 (0.00)	1 (33.33)
OF	4 (7.27)	4 (25)	1 (25)	0 (0.00)
GAT	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
CZ	5 (9.09)	3 (18.75)	1 (25)	0 (0.00)
CN	7 (12.73)	6 (37.5)	0 (0.00)	1 (33.33)
CTX	4 (7.27)	2 (12.5)	0 (0.00)	0 (0.00)
VA	0 (0.00)	1 (6.25)	0 (0.00)	0 (0.00)
COT	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)

Table 5: Antibiotic susceptibility pattern observed in *Streptococci* isolates.

Antibiotics used	CONS	<i>Enterococcus spp</i>	<i>S. aureus</i>	MRSA isolates Resistant; n/N (%)
	n = 25	n = 7	n = 143	
	R (%)	R (%)	R (%)	
AK	4 (16)	3 (42.86)	27 (18.88)	1/5 (20)
TB	3 (12)	2 (28.57)	29 (20.28)	3/5 (60)
G	2 (8)	2 (28.57)	11 (7.69)	2/5 (40)
C	3 (12)	1 (14.29)	27 (18.88)	2/5 (40)
CIP	8 (32)	4 (57.14)	75 (52.45)	4/5 (80)
OF	5 (20)	3 (42.86)	60 (41.96)	3/5 (60)
GAT	0 (0.00)	1 (14.29)	16 (11.19)	1/5 (20)
CZ	5 (20)	1 (14.29)	35 (24.48)	4/5 (80)
CN	12 (48)	4 (57.14)	68 (47.55)	4/5 (80)
CTX	5 (20)	1 (14.29)	53 (37.06)	4/5 (80)
VA	0 (0.00)	0 (0.00)	5 (3.49)	3/5 (60)
COT	1 (4)	1 (14.29)	32 (22.38)	1/5 (20)
DO	-	-	9 (6.29)	1/5 (20)
AZM	-	-	16 (11.19)	0/5 (0.00)
AMX	-	-	35 (24.48)	4/5 (80)
IPM	-	-	0 (0.00)	0/5 (0.00)
CX	-	-	5 (3.49)	5/5 (100)
OX	-	-	5 (3.49)	5/5 (100)

Table 6: Antibiotic susceptibility pattern observed in Staphylococci and Enterococci isolates.

Note: Ak: Amikacin; TB: Tobramycin; G: Gentamicin; C: Chloramphenicol; CIP: Ciprofloxacin; OF: Ofloxacin; GAT: Gatifloxacin, CZ: Cefazolin; CN: Cephalexin; CTX: Cefotaxime; VA: Vancomycin; COT: Cotrimoxazole; DO: Doxycycline Hydrochloride; AZM: Azithromycin; AMX: Amoxycillin; IPM: Imipenem; CX: Cefoxitin; OX: Oxacillin.

The MDR pattern of bacterial isolates

Out of 267 bacterial isolates, 37.08% (n = 99) of isolates were found to be MDR. Among all Gram-positive isolates *Staphylococcus aureus* was found to have highest percent of MDR strains i.e. 48.95% of total *Staphylococcus aureus* isolates which is 70.70% of total MDR strains in this study. Among Gram negative isolates all isolated strains of *E. coli*, *Serratia spp* and *Haemophilus influenzae* strains were found to be multidrug resistant (Table 7).

MIC of ciprofloxacin on MDR *S. aureus* isolates

Among 39 *S. aureus* isolates, 3 isolates were found to grow in 32 µg/ml, 2 isolates grew in 16 µg/ml, 10 isolates grew in 8 µg/ml, 8 isolates grew on 4 µg/ml. 5 isolates were found to be ciprofloxacin intermediate. Remaining 10 isolates grew on 1 µg/ml or less ciprofloxacin concentration (Figure 2).

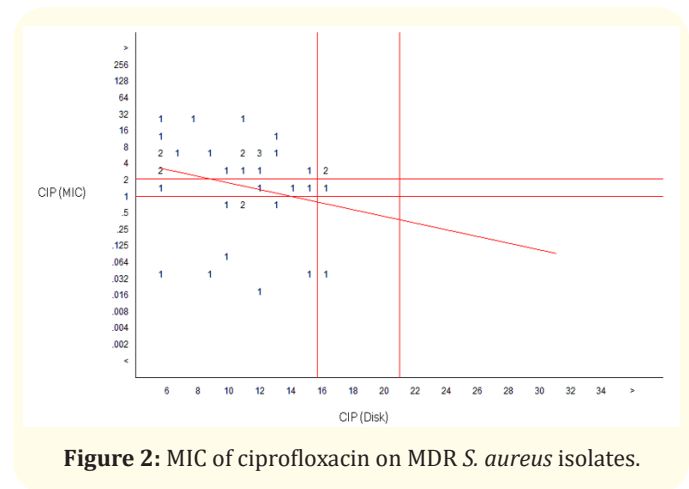


Figure 2: MIC of ciprofloxacin on MDR *S. aureus* isolates.

MIC of chloramphenicol on MDR *S. aureus* isolates

Among twelve *S. aureus* isolates, 3 isolates were found growing in 32 µg/ml, 4 isolates grew in 4 µg/ml and 5 isolates grew in 2 µg/ml. The break point was found to be 13 mm. Three isolates were found to be chloramphenicol resistant (Figure 3).

MIC of vancomycin among MRSA isolates

Among 5 MRSA isolates, 2 isolates were found to be vancomycin intermediates growing in 4 and 8 µg/ml concentration each. The remaining three isolates grew at 2 µg/ml concentration of vancomycin (Figure 4).

Discussion

In our study, highest volume (94.69%) of samples was obtained from the clinical cases of conjunctivitis. This result is in contrast to that reported by other study [27] as the highest sample dealt in this study was corneal scraps showing higher prevalence of corneal

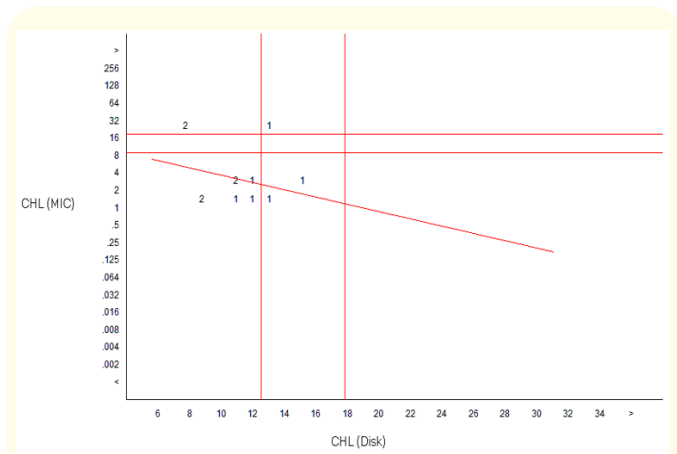


Figure 3: MIC of Chloramphenicol on MDR *S. aureus* isolates.

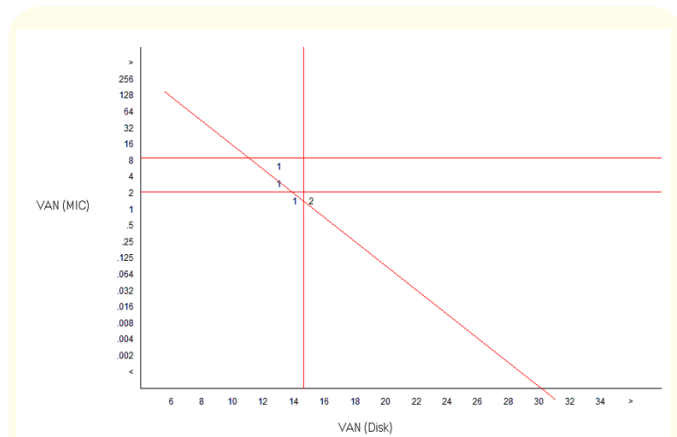


Figure 4: MIC of vancomycin among MRSA isolates.

ulcers. However, in the present study, culture positivity was higher (54.55%) in corneal scraps beside eye pus that showed absolute positivity. Though corneal scraps are referred as sample for few ocular infections, they were more relevant in giving positive results in this study. It is coupled to the fact that there may be only a few organisms in a corneal ulcer, means that adequate clinical material must be obtained in various culture media. As a result, there is traditional practice of taking multiple scrapes from the ulcer and directly plating the material onto several culture media. Hence there are less chances of sampling error and false negative results [28]. Use of direct plating method and enrichment media like BHI for corneal scraps has led to its high positive rate as BHI provides the excellent recovery of fastidious organisms [29].

Here in this study bacterial isolates were in very high proportion as compared to fungal and yeast isolates of which Gram positives were the most frequently isolated ones. This was similar to

Organism Isolated	Drug Resistant Pattern	
	Total (%)	MDR Number (%)
<i>Staphylococcus aureus</i>	143 (53.56)	70 (48.95)
CONS	25 (9.36)	7 (28)
<i>Streptococcus pneumoniae</i>	55 (20.59)	6 (10.90)
<i>Streptococcus pyogenes</i>	3 (1.12)	0 (0.00)
Viridan <i>Streptococci</i>	4 (1.49)	1 (25)
Group D <i>Streptococci</i>	16 (5.99)	3 (18.75)
<i>Enterococcus spp</i>	7 (2.62)	3 (42.86)
<i>Bacillus spp</i>	2 (0.75)	0 (0.00)
<i>Corynebacterium spp</i>	1 (0.37)	0 (0.00)
<i>Micrococcus spp</i>	1 (0.37)	0 (0.00)
<i>Haemophilus influenza</i>	2 (0.75)	2 (100)
<i>Pseudomonas spp</i>	3 (1.12)	2 (66.67)
<i>Escherichia coli</i>	4 (1.49)	4 (100)
<i>Serratia spp</i>	1 (0.37)	1 (100)
Total	267 (100)	99 (37.08)

Table 7: The MDR pattern of bacterial isolates.

Note: CONS: Coagulase Negative Staphylococci

some previous studies [30,31] whereas is in contrast to others [32]. Bacterial isolates are increasingly becoming imperative both clinically and therapeutically as biological agents of ocular infections throughout the world [32]. In the present study, *S. aureus* was the most frequently isolated bacteria among Gram positive isolates as the highest sample number dealt in this study was conjunctival swab and *S. aureus* is the leading cause of conjunctivitis and is most common cause of conjunctivitis in adults while children are most prone to *H. influenzae* [33]. In this study, *E. coli* was the predominant (40%) among total Gram negatives followed by *Pseudomonas spp* (30%), *Haemophilus influenzae* (20%) and *Serratia spp* (10%). This result was in contrast to different studies which revealed *P. aeruginosa* as the predominant among Gram-negative isolates [34,35].

Antibacterial treatments are cheaper and are readily available and its frequent and widespread use has developed resistance of organisms to many commonly used antibiotics [36]. In this study only 3.49% were found as MRSA. Studies reported that risk factors for incidence of MDR MRSA are inappropriate self-medication with antibiotics by patients, their perception, compliance, physicians prescribing practices and antibiotic use in hospital [37]. Our study reported nearly half of the VISA isolates (2/5) among all MRSA isolates. This finding can be compared with some reports from India [38] while is not in harmony with some other from the same [39].

This study reported on the increasing trend of the number of MDR bacterial isolates (37.08%) showing resistance to more than three different classes of antibiotics. All isolates of *Haemophilus influenzae* and *Escherichia coli* followed by *Pseudomonas* spp (66.67%), *Staphylococcus* spp (48.95%), *Enterococcus* spp (42.86%), Viridan *Streptococci* (25%), Group D *Streptococci* (18.75%), and *S. pneumoniae* (10.90%). and a single *Serratia* spp isolated in study was found to be MDR. Though *E. coli* is rarely found in the normal flora of conjunctiva, they are most commonly encountered as the source of infection in ophthalmic neonatorum [40]. *Pseudomonas* spp is the frequent cause of Gram-negative infections like keratitis that can affect various ocular sites in immunocompromised patients and are often resistant to multiple antimicrobial agents [41]. Number of MDR strain in ocular infection has increased than reported from a previous study in Nepal [27].

In our study, *S. aureus* ATCC 29213 exhibited sensitivity to ciprofloxacin, chloramphenicol and vancomycin while was highly resistant (24/143) or intermediate (5/143) to ciprofloxacin. This finding is in harmony with some previous findings [42,43]. The rapid emergence of ciprofloxacin resistance could be probably due to the indiscriminate and empirical use of these drugs [43,44]. Studies showed that fluoroquinolone resistance in *S. aureus* is widespread, which is mainly due to three mechanisms: mutation in nor A gene encoding a membrane-associated active efflux pump, mutation in *gyrA* and *gyrB* genes encoding DNA gyrase and mutation in *grIA* and *grIB* genes, which encode DNA topoisomerase IV [44].

Similarly, three isolates of *S. aureus* were extremely resistant to chloramphenicol. The finding is similar to previous one [45] and is in contrast to other one [43]. The resistant mechanism on the other hand could be due to the most common mechanism against Beta-lactams i.e. enzymatic inactivation or destruction of the drug molecule, rendering it ineffective at the target site of action [43].

Among 5 MRSA isolates, 2 isolates were found as VISA. Various researchers have reported the prevalence of VISA indicating the potential crisis of effective bactericidal antibiotics against this organism [46,47]. Similar findings on VISA were observed from Nepal [48], India [38,39,49]. The report of VRSA from any part of the world has become an international concern as it could well be the next superbug [49]. It is suggested that topical application to the cornea may achieve a very different concentration and bioavailability in the tissue than the serum levels. Thus, MIC is considered as an important measure for evaluating the potential effectiveness of topically applied antimicrobials in the treatment of diseases like bacterial keratitis [50].

This study can be a reference tool to establish the burden of ophthalmic infections in clinical settings. However, broad based surveillance in community settings in Nepal is still lacking which is felt during our literature survey and also seriously affected our study design. Moreover, our study was limited to phenotypic analysis of AMR excluding molecular identification and characterization of wide sorts of genes such as *mecA* in MRSA, ESBL in Gram negative *Enterobacteriaceae* isolates. We suggest the molecular characterization in future researches as these analyses are rapid, specific and reliable. This study can also be useful to the concerned authorities to review existing health policies and practices thereby discouraging rampant misuse of antibiotics, self-medication practices, poor quality of medication and diagnostic tools, inadequate doses and lastly poor surveillance.

Conclusion

Conjunctivitis is the main ocular infection and Gram-positive bacteria were most prevalent than Gram negatives. MDR is yet again identified as the major threat to modern empirical regimens. Of the isolated *S. aureus*, MRSA and VISA is performed which implied that the vancomycin followed by gatifloxacin and gentamycin could be the most efficient drugs for Gram positive isolates and cotrimoxazole, imipenem and piperacillin/ tazobactam for Gram negatives. However, their ocular toxicity needs to be determined. From this study we can also conclude that fungal infection is among the most sight threatening complication and its early diagnosis and study of their AST pattern is vital in treatment of ocular infections due to fungal etiology. Beside all these results this study emphasizes on proper management of empirical treatment and further management of MDR isolates.

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Availability of Data and Materials

All data pertaining to this study are within the manuscript.

Author's Contributions

Samina Thapa and Nabaraj Adhikari have equally contributed to this study. All the authors made substantial contribution to the study. They read and approved the final manuscript.

Consent for Publication

Not applicable.

Competing Interests

All the authors declared that they have no competing interests.

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