Azithromycin, fluoroquinolone and chloramphenicol resistance of non-chlamydia conjunctival bacteria in rural community of Ethiopia

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Aim: To determine profiles of non-chlamydia conjunctival bacteria and their antimicrobial susceptibility from adults who underwent trachomatous trichiasis surgery in rural areas of Ethiopia. **Materials and Methods:** A cross-sectional study was conducted in rural districts in West Gojjam administrative zone. Conjunctival swabs were collected during surgery and transported using Stuart transport broth (Oxoid, UK). Antibiotic susceptibility of conjunctival isolates was determined using the Kirby-Bauer disc-diffusion method. **Results:** Non-chlamydia pathogenic bacteria were recovered from conjunctiva of 438 (31%) participants before treatment. The isolated conjunctival bacteria were *Staphylococcus aureus*, coagulase-negative *Staphylococci*, *Streptococcus* group (A, C, F and G), *Enterococci*, *Streptococcus pneumoniae*, *Moraxella* spp., *Escherichia coli*, *Citrobacter* spp., *Proteus* spp., *Klebsiella* spp., *Pseudomonas* spp. and *Enterobacter* spp. Overall, resistance rates of 57.8% to azithromycin and 68.5% to chloramphenicol were found. However, 86-94.4% sensitivity was demonstrated to ciprofloxacin and norfloxacin. Moderate sensitivity rates (61.8-78.4%) were observed to ceftriaxone, tetracycline and cotrimoxazole. **Conclusion:** Fluoroquinolones that have activity against the majority of bacterial isolates were potent at *in vitro*. However, unacceptably high levels of resistance to azithromycin and chloramphenicol in rural community indicated a need for further study and antimicrobial resistance surveillance.

Key words: Azithromycin, chloramphenicol, fluoroquinolone, non-chlamydia conjunctival bacteria, Ethiopia

A number of studies have demonstrated that arrays of non-chlamydia bacterial pathogens promote inflammation of conjunctival lining of eyelids (trichiasis).[1,2] Topical antibacterial agents such as azithromycin, chloramphenicol, tetracycline and norfloxacin have been prescribed to minimize the risk of conjunctival infection following surgery.[3] Mass azithromycin treatment has been reducing this infection.[4] The World Health Organization (WHO) has begun worldwide program to eradicate blinding trachoma by the year 2020 using mass distribution azithromycin.[5] However, there is a concern that large-scale distribution of azithromycin may lead to the spread of azithromycin-resistant bacterial flora.[6] Therefore, the study was aimed at determining the ocular pathogens and trends of antimicrobial resistance particularly to azithromycin, fluoroquinolon and chloramphenicol in rural community.

Materials and Methods

Conjunctival swabs were collected from adult patients, who underwent trachomatous trichiasis surgery before treatment in rural communities in West Gojjam, Ethiopia from November, 2009 to February, 2010. Conjunctival swabs were collected using sterile Dacron swab during surgery by ophthalmic nurse and physician. Eye swabs were transported using Stuart transport broth (Oxoid, UK) to the Bahir Dar Regional Health Research Laboratory within 24 hours.

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Conjunctival swabs were inoculated onto 5% sheep's blood agar, mannitol salt agar and MacConkey agar plates (Oxoid). The plates were incubated at 37°C aerobically (MacConkey agar, mannitol salt agar) and in an atmosphere of 5% carbon dioxide (5% sheep's blood agar). Urea 40% broth, Simmons citrate, motility-indole medium, Triple Sugar Iron Agar (TSI) and Kligler's Iron Agar (KIA) (Oxoid) were used for differentiation of the Enterobacteriaceae. Phenotypic bacterial identification was made manually in accordance with guidelines of the microbiology standards using colony appearance on culture, Gram staining, biochemical and serology methods.[7]

Morphologically identical 4 to 6 bacterial colonies from overnight culture were suspended in 5 ml Mueller-Hinton broth and incubated for 4 hours at 37°C. Turbidity of the broth culture was equilibrated to match 0.5 McFarland standards. Antimicrobial susceptibility test were performed on Mueller-Hinton agar and 5% sheep's blood plus Mueller-Hinton agar for *Streptococcus* spp. using disk diffusion method.[7] Ciprofloxacin (5 μg), ceftriaxone (30 μg), norfloxacin (10 μg), chloramphenicol (30 μg), gentamicin (10 μg), tetracycline (30 μg), azithromycin (15 μg), cotrimoxazole (25 μg) ampicillin (10 μg) and amoxycillin (10 μg) (Oxoid) were used. Resistance and susceptibility data were interpreted according to National Committee for Clinical Laboratory Standards.[8] Reference strains *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were used as controls. Ethical approval was secured from Research Ethics Committee of Bahir Dar University. Individual verbal consent was obtained from each participant to take conjunctival swab before treatment.

Results

Non-chlamydia pathogenic bacteria were recovered from conjunctiva of 438 (31%) participants. Bacterial species isolated

from adults with trichiasis include *S. aureus*, coagulase-negative *Staphylococci*, *Streptococcus* group (A, C, F and G), Enterococci, *S. pneumoniae*, *Moraxella* spp., *E. coli*, *Citrobacter* spp., *Proteus* spp., *Klebsiella* spp., *Pseudomonas* spp. and *Enterobacter* spp.

Table 1 shows antimicrobial susceptibility patterns of Gram-positive and negative bacteria. Overall, the highest level of resistance was displayed to chloramphenicol, followed by azithromycin, cotrimoxazole, tetracycline and ceftriaxone. However, the lowest levels of resistance were found to ciprofloxacin, norfloxacin and gentamicin [Fig. 1]. All Gram-negative bacteria tested for antimicrobial susceptibility except *Moraxella* spp. were susceptible to ciprofloxacin. Azithromycin was tested against 420 bacterial isolates and 243 (57.8%) showed

resistance. Overall, azithromycin-resistant bacteria revealed higher levels of resistance to other class of antimicrobials than azithromycin-sensitive pathogenic bacteria [Fig. 2].

From Gram-positive bacteria, *S. aureus*, coagulase-negative *Staphylococci* and *Streptococcus* spp. demonstrated high levels of resistance (>80%) to chloramphenicol. Enterococci showed high levels of resistance (72-100%) to tetracycline, azithromycin and ampicillin. Both *S. aureus* and coagulase-negative *Staphylococci* revealed 66-80% resistance to ampicillin and 57-66% to amoxycillin [Table 1]. For Gram-negative bacteria, high levels of resistance were found to cotrimoxazole, chloramphenicol and azithromycin.

Table 1: Antimicrobial resistance patterns of non-chlamydia bacteria from conjunctival swabs in rural community, Ethiopia, 2010

Bacteria	Antimicrobial agents N (%)									
	AZT	C	TE	SXT	Amp	Amox	Cef	G	Nor	Cip
Gram-positive										
S. aureus (*n* = 120)	70 (58)	96 (80)	25 (21)	46 (38)	79 (66)	68 (57)	36 (30)	20 (16.6)	30 (25)	16 (13)
CNS (*n* = 110)	68 (61.8)	89 (81)	60 (54)	46 (41.8)	88 (80)	73 (66)	44 (40)	8 (7.2)	16 (14.5)	*
Streptococcus spp (*n* = 40)	25 (62.5)	32 (80)	22 (55)	13 (32.5)	ND	ND	6 (15)	16 (40)	*	*
S. pneumoniae (*n* = 25)	7 (28)	8 (32)	2 (8)	11 (44)	ND	ND	1 (4)	2 (8)	1 (4)	1 (4)
Enterococci spp (*n* = 25)	20 (80)	ND	18 (72)	ND	25 (100)		ND	2 (8)	1 (4)	3 (25)
Total	190 (59.4)	225 (76.3)	127 (39.6)	116 (39.3)	192 (75)	141(61)	87 (29.5)	48 (15)	48 (15)	20 (6.2)
Gram-negative										
Moraxella spp. (*n* = 15)	11 (73)	8 (53.3)	6 (40)	12 (80)	ND	ND	6 (40)	6 (40)	8 (53.3)	6 (40)
E. coli (*n* = 20)	11 (55)	7 (35)	5 (25)	3 (15)	ND	ND	*	4 (20)	*	*
Citrobacter spp. (*n* = 20)	10 (50)	10 (50)	2 (10)	10 (50)	ND	ND	*	*	*	*
Proteus spp. (*n* = 25)	11 (55)	19 (76)	14 (56)	7 (28)	ND	ND	*	2 (8)	*	*
Klebsiella spp. (*n* = 20)	10 (50)	11 (55)	*	7 (28)	ND	ND	4 (20)	*	*	*
Pseudomonas spp. (*n* = 20)	*	10 (50)	4 (20)	10 (50)	ND	ND	3 (15)	*	1 (5)	*
Enterobacter spp. (*n* = 20)	*	8 (40)	5 (25)	6 (30)	ND		1 (5)	4 (20)	*	*
Total	53 (53)	73 (52.1)	36 (25.7)	55 (55)	ND	ND	14 (10)	16 (11)	9 (6.4)	6 (4.3)

CNS: Coagulase negative staphylococci, AZT: Azithromycin, C: Chloramphenicol, TE: Tetracycline, Amp: Ampicillin, Amox: Amoxycillin, Cef: Ceftriaxone, G: Gentamicin, Nor: Norfloxacin, Cip: Ciprofloxacin, *All are susceptible, ND: Not done
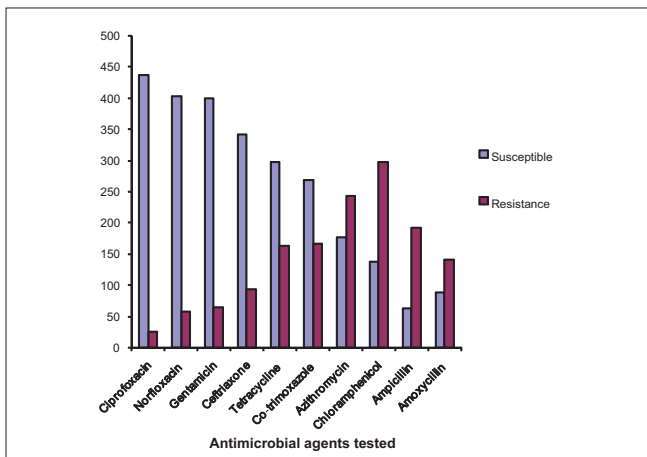

Figure 1: Susceptibility and resistance profile of non-chlamydia bacteria isolated from conjunctival swabs from rural community, Ethiopia, 2010
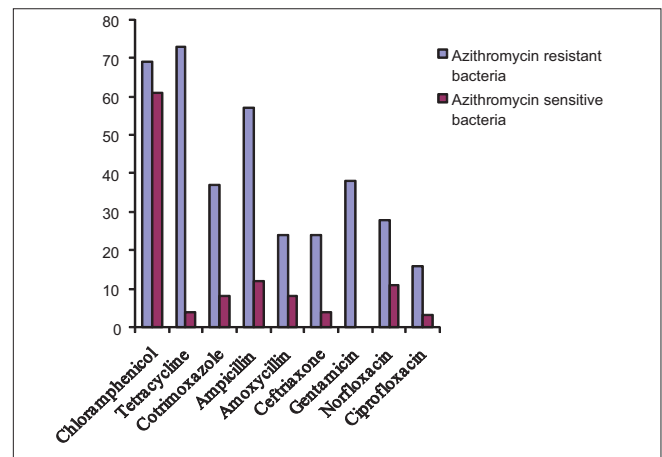

Figure 2: Antimicrobial resistance patterns of azithromycin resistant and sensitive bacteria against other class of antimicrobials resistance, Ethiopia, 2010

Discussion

The non-chlamydia conjunctival bacteria isolated in this study were *S. aureus*, coagulase negative Staphylococci spp., *Streptococcus* spp., *S. pneumoniae*, *Proteus* spp., *E. coli*, *Klebsiella* spp., *Citrobacter* spp., *Pseudomonas* spp., *Enterobacter* spp. and *Moraxella* spp. This is result is in agreement with the findings of other studies in Africa.[1,2] However, Chern *et al*.[9] from Nepal found that the most frequently isolated non-chlamydia bacteria were *S. pneumoniae*, *Hemophillus influenza* and *Moraxella* spp and with less frequency of Gram-negative rods such as *E. coli*.

We speculated low level of antimicrobial resistance patterns for bacterial isolates from rural communities than clinical isolates. However, this study demonstrated high azithromycin resistance (57.8 %) compared to clinical isolates reported from Turkey and USA.[10,11] However, Mendes *et al*.[12] from Brazil reported 8-36% azithromycin resistance for Gram-positive cocci.

In this study, at baseline higher rate of azithromycin-(28%) resistant *S. pneumoniae* were detected compared to other studies. Leach *et al*.[13] reported 1.9 % *S. pneumoniae* azithromycin resistance at baseline from Australia. However, following azithromycin treatment, significantly higher azithromycin-resistant *S. pneumoniae* were detected. A study conducted in Australia found 55% azithromycin-resistant *S. pneumoniae* after 2 -3 weeks and in Nepal 42.8% resistant rate after 2 weeks.[13,14]

The possible explanation for high resistance to azithromycin in the present study at baseline could be the selective pressure of azithromycin mass treatment. Since, mass community-wide distributions of azithromycin have been practiced in Ethiopia since 2003.[15] Thus this might enable rapid expansion of resistant strains through cross-transmission. However, Hong *et al*.[16] reported non-azithromycin-resistant *C. trchomatis* in Ethiopia.

Regarding chloramphenicol, very high levels of resistance to the majority of bacterial isolates from rural communities were observed [Fig. 1]. Specifically, S. *aureus*, *Streptococcus* spp., *Proteus* spp., *Klebsiella* spp. and *Citrobacter* spp. revealed higher resistance levels compared to their respective clinical isolates from previous studies from Ethiopia.[17,18] Chloramphenicol-resistant bacteria might be acquired through cross-contamination from close contact with domestic animals. This can be supported by a study conducted in Nigeria indicating that antimicrobial-resistant *Staphylococci* from animals were associated with the cause of wound infections in outpatients.[19] Moreover, in developing countries, people with close contact with domestic animals have chance of acquiring resistant microbes from animals.[20] High level of chloramphenicol resistance in rural communities could be due to indiscriminate use of antibiotics by rural communities.[21] However, further systematic surveillance need to be carried out to elucidate this concern.

Fluoroquinolones are critically important antimicrobials in treatment of human infections. Overall, lowest levels of resistance were observed to ciprofloxacin and norfloxacin. From all tested bacteria *S. aureus* and *Moraxella* spp. displayed highest fluoroquinolone resistance. Abera *et al*.[22] found high ciprofloxacin resistance levels to methicillin-resistant *S. aureus* (MRSA) at local hospital near the present study area. Currently, fluoroquinolones particularly ciprofloxacin

is prudent for empirical treatments of community infections in rural communities.

Enterococci become an increasingly important cause of nosocomial infections most frequently in urinary tract infections, endocarditis and wound infections. In this study, *Enterococci* from conjunctival swabs showed high levels of resistance (72-100%) to ampicillin, azithromycin and tetracycline. This conforms to the results of clinical isolates from other study in Jimma hospital, Ethiopia.[18] However, Simonsen *et al*.[23] indicated that no ampicillin resistance to *E. faecalis* and 48.8% ampicillin resistance to *E. faecium* from hospitals in Norway.

In conclusion, unacceptable levels of azithromycin and chloramphenicol resistance to an array of non-chlamydia conjunctival bacteria from rural communities were observed. However, ciprofloxacin, norfloxacin and gentamicin would be prudent for empirical treatment of community-acquired infections. This preliminary finding indicated a need for further study and antimicrobial resistance surveillances.

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