ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF BACTERIAL AND FUNGAL OCULAR ISOLATES FROM TERTIARY CARE OPHTHALMIC HOSPITALS IN CHENNAI

Senthilvadivu C¹, Stalin M²

¹Assistant Professor, Department of Microbiology, Government Dharmapuri Medical College, Dharmapuri, Tamilnadu.

²Tutor, Department of Microbiology, Government Dharmapuri Medical College, Dharmapuri, Tamilnadu.

ABSTRACT

BACKGROUND

Infectious keratitis is an ophthalmic emergency that needs immediate institution of treatment. Recent studies have shown increasing evidence of resistance of microbes to antimicrobial agents, which have further added to the reasons for poor prognosis in microbial keratitis.

Aims and Objectives- This study was carried out to understand the antimicrobial susceptibility pattern of bacterial and fungal isolates in keratitis.

MATERIALS AND METHODS

A total of 150 corneal scraping samples were collected and identified by standard protocol and were tested for antibiotic susceptibility as per CLSI guidelines with various groups of antibiotics. MIC for vancomycin, amphotericin B and itraconazole were determined for study isolates by agar dilution method.

RESULTS

Out of 150 corneal scraping samples, 97 (64.6%) showed culture positivity. Among bacterial, fungal and parasitic agents isolated fungi were the predominant aetiological agents. *Staphylococcus aureus* (26.4 %), *Aspergillus* sp. and (24.8 %) *Acanthamoeba* sp. were the most frequently isolated bacterial, fungal and protozoan aetiological agents respectively. Majority of the bacterial isolates were susceptible to amikacin (94.1%) and were least susceptible to cotrimoxazole (26.4%). All *Staphylococcus* sp. were found to be susceptible to vancomycin with MIC range of 0.25 - 0.5 µg/mL. More than 50% of fungal isolates were susceptible to both amphotericin B and itraconazole and none of the fungal isolates were susceptible to fluconazole. About 85.9% of fungal isolates had MIC value of (MIC < 2 µg/mL) for itraconazole.

CONCLUSION

This study suggests that the in vitro susceptibility testing of bacterial and fungal isolates of infectious keratitis is extremely useful in clinical management and should be performed as a routine diagnostic protocol.

KEYWORDS

Infectious Keratitis, Antimicrobial Susceptibility and MIC.

HOW TO CITE THIS ARTICLE: Senthilvadivu C, Stalin M. Antimicrobial susceptibility pattern of bacterial and fungal ocular isolates from tertiary care ophthalmic hospitals in Chennai. J. Evolution Med. Dent. Sci. 2018;7(15):1868-1871, DOI: 10.14260/jemds/2018/421

BACKGROUND

Corneal ulcer or infectious keratitis is a serious condition of cornea that requires prompt management. It can be caused by bacteria, virus, fungus, protozoa and parasites. Varieties of antibiotics are available in the market for the treatment of ocular infections and administered by various routes including topical instillation, subconjunctival injection and intraocular injection of antibiotics. Eye drops are available in fortified forms to achieve higher concentration in ocular tissues. Antibiotic resistance among ocular pathogens is increasing in parallel with the increase of resistance in other systemic pathogens. The factors contributing to development of drug resistance among ocular isolates include overuse of

antibiotics for systemic infection as well as overuse of topical antibiotics in the eye.¹ If the appropriate antimicrobial treatment is delayed, only 50% of the eyes gain good visual recovery.² Though the majority of community acquired cases of microbial keratitis resolves with empiric treatment and do not require culture, corneal scraping for culture and sensitivity is indicated for corneal ulcers that are unresponsive to broad-spectrum antibiotic therapy.³ Recent studies have shown increasing evidence of resistance of microbes to antimicrobial agents.^{1,4} Hence, knowledge of the microbiological pattern of bacterial and fungal keratitis will be helpful for effective management of keratitis in situations where resources are limited. Hence, this study was done to determine the antimicrobial susceptibility profiles of bacterial and fungal aetiological agents among patients with infective keratitis from a tertiary care Regional Institute of Ophthalmology and Rajiv Gandhi Government General Hospital in Chennai.

MATERIALS AND METHODS

A descriptive study was carried out over a period of one year from October 2011 to September 2012. A total of 150 corneal scraping samples were collected from patients presenting in the outpatient department with the history of trauma and

'Financial or Other Competing Interest': None.

Submission 27-02-2018, Peer Review 24-03-2018,

Acceptance 30-03-2018, Published 09-04-2018.

Corresponding Author:

Dr. Senthilvadivu C,

Assistant Professor,

Department of Microbiology,

Government Dharmapuri Medical College,

Dharmapuri, Tamilnadu.

E-mail: ezhilan14@yahoo.com

DOI: 10.14260/jemds/2018/421

signs and symptoms of infectious corneal ulcer. Bacterial, fungal and protozoan isolates were identified by standard protocol and were tested for antibiotic susceptibility by the Kirby-Bauer disc diffusion method on Mueller Hinton agar (HiMedia) and were interpreted in accordance with the CLSI guidelines.⁵ Methicillin resistance was detected by taking oxacillin disc (1 µg) as a surrogate marker. Antibigram was done with the following antibiotics: Amikacin (30 µg), cefotaxime (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), cotrimoxazole (25 µg), erythromycin (15 µg), imipenem (10 µg), ofloxacin (5 µg) and penicillin G (10 U). MIC for vancomycin was determined by agar dilution method as per CLSI guidelines.⁵ Antifungal susceptibility disc diffusion test was performed on Mueller-Hinton agar plates supplemented with 2% Glucose and 0.5 µg/mL of methylene blue and the following antifungal agents: Amphotericin B (100 U), itraconazole (10 µg) and fluconazole (10 µg). MIC for amphotericin B and itraconazole were determined by agar dilution method. The reference strains used were *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Aspergillus flavus* ATCC 204304 and *Aspergillus fumigatus* ATCC 204305.

RESULTS

Out of 150 corneal scrapings, 97 (64.6%) samples were found to be culture positive. Among bacterial, fungal and parasitic agents isolated, fungi 57 (38%) were the predominant aetiological agent. *Staphylococcus aureus* (26.4%), *Aspergillus sp.* (24.8%) were the most frequently isolated among bacterial and fungal agents respectively and *Acanthamoeba sp.* was the only parasitic agent isolated in infectious keratitis (Table 1). Majority of the bacterial isolates were susceptible to amikacin (94.1%) followed by ciprofloxacin (82.3%), cefotaxime (79.4%), ofloxacin (61.7%) and were least susceptible to gentamicin (44.1%) and cotrimoxazole

(26.4%) (Table 2). Methicillin resistance was observed among *S. aureus* 2/9 (22.2%) and *S. epidermidis* 2/6 (33.3%). All *Staphylococcus sp.* were found to be susceptible to vancomycin by agar dilution method with MIC range of 0.25-0.5 µg/mL. More than 50% of fungal isolates were susceptible to both amphotericin B and itraconazole and none of the fungal isolates were susceptible to fluconazole (Table 3) and (Plate No. 1). More than 66% of fungal isolates had MIC value of (MIC < 2 µg/mL) for amphotericin B (Table 4). Out of 59, 47 (85.9%) fungal isolates had MIC value of (MIC ≤ 2 µg/mL) for itraconazole by agar dilution method (Table 5).

Bacterial Agents	No. of Isolates (%)
<i>Staphylococcus aureus</i>	9 (26.4%)
<i>Staphylococcus epidermidis</i>	6 (17.6%)
<i>Pseudomonas aeruginosa</i>	6 (17.6%)
<i>Klebsiella pneumoniae</i>	5 (14.7%)
<i>Acinetobacter baumannii</i>	4 (11.7%)
<i>Escherichia coli</i>	4 (11.7%)
Total	34 (100%)
Fungal Agents	
<i>Aspergillus flavus</i>	14 (24.5%)
<i>Aspergillus niger</i>	12 (21%)
<i>Aspergillus fumigatus</i>	10 (17.5%)
<i>Fusarium spp.</i>	12 (21%)
<i>Curvularia spp.</i>	2 (3.5%)
<i>Penicillium spp.</i>	3 (5.2%)
<i>Graphium spp.</i>	2 (3.5%)
<i>Scopulariopsis spp.</i>	2 (3.5%)
Total	57 (100%)
Parasitic Agents	
<i>Acanthamoeba spp.</i>	6 (100%)
Table 1. Distribution of Bacterial, Fungal and Parasitic Agents among Infectious Keratitis Patients	

Organism	Total No.	Amikacin		Gentamicin		Ciprofloxacin		Ofloxacin		Cefataxime		Cotrimoxazole		Cephalexin		Vancomycin		Oxacillin	
<i>Staphylococcus aureus</i>	9	9	100%	-	-	7	77%	7	77%	8	88%	5	55.5%	7	77%	9	100%	7	77%
<i>Staphylococcus epidermidis</i>	6	6	100%	-	-	6	100%	-	-	6	100%	4	66%	4	66%	6	100%	4	66%
<i>Pseudomonas aeruginosa</i>	6	6	100%	5	83%	4	66%	5	83%	4	66%	-	-	5	83%	-	-	-	-
<i>Klebsiella pneumoniae</i>	5	5	100%	4	80%	5	100%	4	80%	3	60%	-	-	4	80%	-	-	-	-
<i>Acinetobacter boumanii</i>	4	3	75%	3	75%	2	50%	2	50%	3	75%	-	-	3	75%	-	-	-	-
<i>Escherichia coli</i>	4	3	75%	3	75%	4	100%	3	75%	3	75%	-	-	3	75%	-	-	-	-
Percentage of Sensitivity	34		94.11%		44.11%		82.35%		61.76%		79.41%		26.47%		76.47%		100%		32.35%

Table 2. Antimicrobial Susceptibility Pattern of Bacterial Isolates of Infectious Keratitis

Agent	No. of isolates	Amphotericin-B (20µg) S>15 mm	Itraconazole (10µg) S>23 mm	Fluconazole (10µg) S>19 mm
<i>Aspergillus flavus</i>	14	9 (64%)	11 (78%)	R
<i>Aspergillus niger</i>	12	7 (58%)	12 (100%)	R
<i>Aspergillus fumigatus</i>	10	7 (70%)	10 (100%)	R
<i>Fusarium spp</i>	12	8 (66%)	10 (83%)	R
<i>Curvularia spp</i>	2	1 (50%)	1 (50%)	R
<i>Penicillium spp</i>	3	1 (33%)	2 (66%)	R
<i>Graphium spp</i>	2	1 (50%)	2 (100%)	R
<i>Scopulariopsis spp</i>	2	1 (50%)	2 (100%)	R

Table 3. Antibiotic Susceptibility Pattern of Fungal Isolates by Disc Diffusion Method

Organism	0.25 µg	0.5 µg	1 µg	2 µg	4 µg	8 µg	16 µg	32 µg	64 µg
<i>Aspergillus Flavus</i>	3	2	5	2	1	-	1	-	-
<i>Aspergillus niger</i>	2	3	3	1	1	1	1	-	-
<i>Aspergillus Fumigatus</i>	1	3	1	2	1	1	-	1	-
<i>Fusarium spp</i>	3	1	3	3	1	1	-	-	-
<i>Curvularia spp</i>	-	1	1	-	-	-	-	-	-
<i>Penicillium spp</i>	-	1	1	-	1	-	-	-	-
<i>Graphium spp</i>	-	1	1	-	-	-	-	-	-
<i>Scopulariopsis spp</i>	-	-	1	1	-	-	-	-	-

Table 4. MIC for Amphotericin B by Agar Dilution Method

Organism	0.25 µg	0.5 µg	1 µg	2 µg	4 µg	8 µg	16 µg	32 µg	64 µg
<i>Aspergillus Flavus</i>	3	4	2	4	1	-	-	-	-
<i>Aspergillus niger</i>	2	3	2	3	1	1	-	-	-
<i>Aspergillus Fumigatus</i>	-	2	3	2	1	-	-	1	1
<i>Fusarium spp</i>	2	3	2	3	1	1	-	-	-
<i>Curvularia spp</i>	1	-	1	-	-	-	-	-	-
<i>Penicillium spp</i>	1	-	1	1	-	-	-	-	-
<i>Graphium spp</i>	-	1	1	-	-	-	-	-	-
<i>Scopulariopsis spp</i>	-	-	1	1	-	-	-	-	-

Table 5. MIC for Itraconazole by Agar Dilution Method

Plate No. 1. Antifungal Susceptibility by Disc Diffusion Method

DISCUSSION

Microbial resistance to antibiotic agents is becoming increasingly prevalent in ocular infections. Immediate diagnosis and treatment are important to avoid vision-threatening outcomes including corneal scarring or perforation.⁶ The past two decades have witnessed changes in antibiotic susceptibility patterns on a worldwide basis. Guidelines that have been developed to help slow the escalation of systemic antibiotic resistance and encourage prudent use of antibiotic agents also apply to the management of ocular infections. In this study, fungal agents were the most common agents isolated in infectious keratitis patients followed by bacterial and parasitic agents. This is in accordance with a study by Khanal *et al* (2005) in Nepal who reported 42.7% culture positive for fungal agents.⁷ In this study, *Aspergillus sp.* was the predominant isolate (63.1%) followed by *Fusarium sp.* (21%) and this correlates with study from Hyderabad by Gopinathan *et al* (2002).⁸ Among the bacterial isolates *Staphylococcus aureus* 9 (27%) was the most common bacterial agent isolated, which was similar to the study by Basak *et al* (2005) who reported that *Staphylococcus aureus* was the most common bacterial isolate.⁹ Among the parasitic agents protozoa like *Acanthamoeba sp.* was isolated in 6 patients (6.3%) and this was the only parasitic agent isolated. Corneal infection due to *Acanthamoeba* is believed to result from direct corneal contact with contaminated material or water. Various Indian studies show prevalence rate of 1% to 4% *Acanthamoeba* culture positive corneal ulcers.¹⁰ Amikacin was found to be the most effective antibiotic (94.1%) for the bacterial isolates in this study. This coincides with a study by Fong *et al* (2007).¹¹ Susceptibility to ofloxacin and gentamicin antibiotics were comparatively lower in this study indicating the selective usage for the treatment in the study sites. In this study, 22.2% of *S. aureus* isolates were found to be methicillin resistant. In this study both gram positive and gram negative bacterial isolates showed susceptibility to amikacin, which was similar to the study report of Kaliamurthy *et al* (2013).¹² High percentages of fungal isolates were susceptible to both amphotericin B and itraconazole in this study suggesting that they have effective role in management of fungal keratitis. Alarmingly, all the fungal isolates were found to be resistant to fluconazole, indicating no longer it can be used for the empirical therapy. Management of fungal keratitis remains a challenge to cornea specialists. Current selection of antifungals is based on animal experiments, clinical experience and published sensitivity data. *In vitro* sensitivity testing of a particular isolate is extremely useful and should be performed whenever its availability is not a concern.¹³ In this study, majority of fungal isolates (66%) had MIC value of (< 2 µg/mL) for amphotericin B and 85.9% of fungal isolates had MIC value of (≤ 2 µg/mL) for itraconazole, strongly suggesting that it has a vital role in management of fungal keratitis.

CONCLUSION

This study suggests that emerging fungal pathogens and resistance to existing antifungal drugs demand the importance of antifungal susceptibility testing to manage fungal keratitis and the clinicians should prescribe antibiotic agents only when clearly indicated and should order susceptibility testing whenever possible to prescribe the

most appropriate antibiotic to avoid development of antibiotic resistance.

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