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Full Length Research Paper

Bacteria isolated from contact and non contact lens and antibiotic susceptibility patterns of isolated *Pseudomonas aeruginosa*

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The aim of this study was to investigate the prevalence and type of microbial contamination, associated with contact lenses and lens care accessories used by a group of contact lens wearers. Results show that a total of 178 strains were isolated, including, 100 Gram positive and 78 Gram negative bacteria. *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* were the most common microorganisms isolated (25.281 and 13.483% respectively. 24 *P. aeruginosa* were isolated from lenses and eyes of contact lens and non-contact lens wearers. All isolates were susceptible to the tested aminoglycosides and fluoroquinolones. Aminoglycosides and fluoroquinolones (ciprofloxacin) were more efficient than *β*-lactams. 91.67% of the strains had intermediate-resistant to cefotaximee; 4.17% were resistant and 4.17% were sensitive to Cefotaximee. 95.83% of the strains were sensitive, while 4.17% were resistant to Imipenem. 20.83 and 12.5% of *P. aeruginosa* strains were resistant and sensitive to Ceftriaxone respectively, while 66.67% were moderately-resistant to ceftriaxone of the *β*-lactam class. All isolates were sensitive to the other tested *β*-lactam.

Key words: Microbial keratitides, *Pseudomonas aeruginosa*, contact lenses.

INTRODUCTION

For more than 20 years, many researchers have worked toward understanding why the corneas of contact lens wearers are more susceptible to infection (Evans et al., 2007; Willcox, 2007; Pearlman et al., 2008; Fleiszig et al, 2006; Maltseva, 2007 and Fleiszig and Evans, 2010). Several decades of research and some major advances in lens and solution technology have not resulted in a decline in disease incidence (Fleiszig and Evans, 2010).

Contact lens wear continues to be a significant risk factor for the development of acute sight-threatening corneal infections (microbial keratitis) as reported (Green et al., 2008a; Ibrahim et al., 2009; Edwards et al., 2009; Stapleton et al., 2008). Devonshire et al. (1993) reported that the problem in contact lens wear was the presence of bacteria and other microorganisms; because some contact lens wearers had developed microbial keratitis. Martins et al. (2002) observed the presence of fungi, parasites and bacteria in contact lens swabs cultures. It has been reported that the environment, the type of contact lens (CL), the duration of wear, and the type of CL cleansing solution determined the microbial load on the contact lenses (Iskeleli et al., 2002; Lee and Lim, 2003). *Staphylococcuss epidermidis, Staphylococcuss aureus, Enterobacter* and *Pseudomonas* species found in healthy eyes, were also observed on soft contact lenses of healthy persons (Sankaridurg et al., 2000).

Pseudomonas aeruginosa is a Gram-negative, opportunistic pathogen implicated in sight-threatening ocular infectious diseases such as keratitis (Sharma et al., 2006; Willcox, 2007; Green et al., 2008a).

P. aeruginosa keratitis is considerably more common in contact lens wearers compared with non-contact lens wearers, presumably because of the altered ocular environment. Bacterial contamination of lenses and storage cases has been reported even in association with good compliance with care and hygiene regimens. Phenotypic traits expressed in biofilms are partially responsible for the emerging resistance against

antimicrobial therapy (del Pozo and Patel, 2007) of contact lens-related keratitis. In addition, emergence of multi-drug resistance in *P. aeruginosa* strains (Rossolini and Mantengoli, 2005) becomes a major concern when antibiotics such as fluoroquinolones are used as monotherapeutic agents.

P. aeruginosa is also one of the most commonly cultured organisms in non-contact lens-related ocular trauma events that lead to keratitis (Hooi and Hooi, 2005; Parmar et al., 2006; Green et al., 2008b). In addition, emergence of multi-drug resistance in *P. aeruginosa* strains (Rossolini and Mantengoli, 2005) becomes a major concern when antibiotics such as fluoroquinolones are used as monotherapeutic agents (Choy et al., 2008). From their results, they suggest that *P. aeruginosa* isolates from different infection origins may have different characteristics. Multi drug resistance in *P. aeruginosa* is steadily increasing also worldwide (Navon-Venezia et al., 2005). Although definitions of multi drug resistance are variable, they often involve resistance to fluoroquinolones, expanded-spectrum cephalosporins, carbapenems, and aminoglycosides. As an example, a progressive increase in multi drug-resistant *P. aeruginosa* (resistance to ≥3 antibiotics) was observed from 7.1% in 2001 to 9.9% in 2003 in the US (Navon-Venezia et al., 2005; Falagas and Bliziotis, 2007).

There is no reason why such an increase of multi drug resistance (MDR) in *P. aeruginosa* may slow down soon, especially in countries that face a high level of MDR of *P. aeruginosa* (Asia, South America, Southern Europe and countries located on the border of the Mediterranean sea). Multi drug resistance is worrisome since it corresponds to the addition of unrelated mechanisms of resistance that are difficult if not impossible to reverse once gathered in single genetic resistance structures (transposon, integron, plasmid); these latter structures contributes to co-selection of resistances. Recent reports unravel the successive mechanisms (efflux, outer membrane permeability defect) that are at the basis of MDR development in clinical *P. aeruginosa* isolates (Reinhardt et al., 2007; El'Garch et al., 2007). The aim of this study was to investigate the prevalence and type of microbial contamination, identify the contaminants associated with contact lenses and lens care accessories used by a group of contact lens wearers and to evaluate the resistance or susceptibility of *P. aeruginosa,* which is the most common pathogen in contact lens keratitis and corneal ulcer to different antibiotic regimens.

MATERIALS AND METHODS

Clinical isolates were obtained from contact lens storage cases, contact lenses and contact lenses wearer between November 2010 and December 2011 in Saudi Arabia. Contact lenses samples were collected by using sterile cotton swabs moisturized with normal saline solution, while eyes samples were done by swabbing the lower conjunctive sac by sterile cotton swabs. The swabs were incubated in brain heart infusion tubes and incubated for 24 h at 37°C. According to MacFaddin (2000), sub cultures were done on blood agar, MacConkey agar, Cetrimide agar, Vogel-Johnson's agar and nutrient agar and were incubated at 37°C for 24 h. Identification of bacterial isolates were done by Gram's staining, using selective media and biochemical tests including catalase, coagulase, and oxidase test according to Lancette and Tatini (1992). Further identification of enteric organisms was done using the API 20E test strips. *P. aeruginosa* strains were identified by 16S rRNA gene (Al-Zahrani et al., 2012).

P. aeruginosa antibiotics susceptibility tests

Twenty-four (24) *P. aeruginosa* clinical isolates were subjected to antibiotic susceptibility test according to NCCLS (1994) by disc diffusion method; Amikacin, 30 µg; Gentamicin, 10µg; Meropenem, 10 µg; Impienim, 10 µg, Netilmicin, 30 µg; Piperacillin/Tazobactam, 110 µg; Ceftriaxone, 5 µg; Aztreonam, 30 µg; Ceftazidimeug 30 µg; Ciprofloxacin 5 µg; Norfloxacin, 10 µg and Cfotaxim 5 µg. MIC bacterial susceptibilities to these antibiotics were determined using Microscan (walk away 96 SI plus) from Siemens; *P. aeruginosa* ATCC27853 was used as a test control.

RESULTS

Results show that a total of 178 strains were isolated including 100 Gram positive and 78 Gram negative bacteria. The bacterial species were *Acinetobacter baumannii*, *Enterobacter cloacae*, *P. aeruginosa, Enterobacter aerogenes,* isolated from eyes-lenses, *Serratia marcescens*, *Providencia rettgeri*, *Providencia rettgeri*, *Acinetobacter lwoffii, Burkholderia cepacia, Micrococcus luteus* and *Staphylococcuss aureus* from lenses, *Acinetobacter lwoffii*, *Klebsiella pneumoniae* from lenses, lens cases, *Bacillus* sp. and *S. epidermidis* from eyes, lenses, lens care solutions and lens cases (Table 1). From patients with endophthalmitis, for the isolates from contact lenses and isolates from eyes and lenses, *S. epidermidis* was the most common microorganisms found in this study**.**

Before this study, it was reported that *P. aeruginosa* was the most common contaminant of contact lenses but as asymptomatic subjects were analyzed during the study, the results in show that *S. epidermidis* was the highest number of all the isolate 45 (25.28%) (Table 1). Members of transient flora are considered to be of little significance as long as the normal epithelial surface remains intact. It has been implicated in several lens wearer complications including keratitis and corneal ulcers. The results of the present study are reflective of the observation that *P. aeruginosa* and *S. epidermidis* are the dominant bacteria that cause ocular infections among contact lens wearers. These finding are in confirmation with the earlier reports (Kanpolat, 1992).

In this study, Gram^{+} *Bacillus sp.* rate were 15 (8.427%). Few cases of *Bacillus* keratitis among contact lens wearers were reported earlier (Pinna et al., 2001). *Bacillus* spores survived multiple lens disinfection treatments.

Other bacterial isolates were found in small number

Table 1. Bacterial strains isolated from contact lens and non- contact lens wearers.

Gram stain	Number of isolate	Bacteria	Source	Isolate (%)
	10	*Achromobacter xylosoxidans*	Eyes -lenses	5.618
	10	*Acinetobacter baumannii*	Eyse - lenses	5.618
	6	*Enterobacter cloacae*	Eyes - lenses	3.371
	5	*Pseudmonas fluorescens*	Eyes - lenses	2.809
	4	*Enterobacter aerogenes*	Eyes - lenses	2.247
Gram$^-$	24	*Pseudomonas aeruginosa*	Eyes - lenses	13.483
	13	*Serratia marcescens*	Leness	7.303
	4	*Providencia rettgeri*	Lenses	2.247
	8	*Acinetobacter lwoffii*	Lenses	4.494
	8	*Proteus.mirabilis*	Lenses - lens Cases	4.494
	8	*Klebsiella pneumoniae*	Lenses- lens cases	4.494
	15	*Bacillus* sp.	Eyes - lenses-lens care solutions	8.427
	9	*Burkholderia cepacia*	Lenses	5.056
Gram$^+$	4	*Micrococcus luteus*	Lenses	2.247
	5	*Staphylococcus aureus*	Lenses	2.809
	45	*Staphylococcus epidermidis*	Eyes - lenses- lens cases and lens care solutions	25.281
Total	178			100

Such as *Enterobacter cloacae* 6 (3.37%) *Pseudmonas fluorescens,* and *S. aureus* 5 (2.809%) while *Enterobacter aerogenes* and *Micrococcus luteus* were the smallest number 4 (2.247%). *S. epidermidis, S. aureus, Enterobacter* and *Pseudomonas* species found in healthy eyes, were also observed on soft contact lenses of healthy persons (Sankaridurg et al., 2000).

P. aeruginosa isolates were the second highest number of all isolate 24 (13.48%). Results in (Table 2) illustrate the origin of the isolated 24 strains; one of them was isolated from patients with endophthalmitis, and 4 from contact lenses belonging to a patient with contact lens-associated red eye (CLARE) and 12 strains from patients with keratitis strains from consecutive patients attending King Khaled Eye Hospital in Riyadh, Saudi Arabia over a 12-month period. The remaining five strains were isolated from contact lens cases belonging to asymptomatic wearers (CLCaw). Contact lenses made from nonionic polymers with high water content may carry higher risks of bacterial contamination (Dang et al., 2003).

P. aeruginosa was found to be the next dominant organisms. It is a Gram$^-$ rod that is considered as transient microorganisms in the normal healthy eyes. The transient flora is contracted from the environment and inhabits the conjunctiva for hours, days or weeks.

Aminoglycosides and fluoroquinolones (ciprofloxacin) are more efficacious than *β*-lactams. The MIC test results show that 91.67% of strains were intermediately-resistant to Cefotaximee, except two of the isolates (8.33%), strain 7 and strain 23 were resistant and sensitive to Cefotaximee respectively. 95.83% of the strains were sensitive, except strain 3 which was resistant to Imipenem (4.17%). Five isolates (20.83%) of *P. aeruginosa*, strains 6, 8, 17, 22 and 24 were resistant to Ceftriaxone and three strains, 12, 18 and 23 were sensitive (12.5%), while 66.67% were intermediate-resistant to ceftriaxone of the *β*-lactam class. All isolates were sensitive to other tested *β*-lactam.

Table 2. Contact lens-related and non-contact-lens-related *P. aeruginosa*.

Number of isolate	Source	The case of the source
7	Soft contact lenses	Used by healthy persons
4	Soft contact lenses	Patients with contact lens-associated red eyes (CLARE)
1	Eyes of contact lenses wearers	Asymptomatic wearer
12	Eyes of contact lenses wearers	Patients with keratitis

Antibiotic susceptibility test by disc diffusion method results for *P. aeruginosa* are showed in Table (3). Most of the isolates were resistant to Ceftriaxone except isolates 12, 15, 16, 18 and 23 and the control test *P. aeruginosa* ATCC 27853. All isolates except isolate 23 were resistant to Cefotaxime. All isolates were sensitive to the other antibiotics that were used in this study except isolate 3 which was resistant to Impienim.

Table 3. Sensitivity (S) and resistant (R) of *P. aeruginosa* to antibiotics by diffusion method.

Isolate of number	Diameter of inhibition zone (in mm)										
	Cefotaxime	Norfloxacin	Aztreonam	Ceftriaxone	Tazobactam	Netilmicin	Impienim	Meropenem	Gentamicin	Amiacin	Ceftazidime
Control	R	29	27	18	34	24	25	33	22	26	30
1	R	30	25	R	29	22	21	29	20	24	28
2	R	31	27	R	30	24	26	23	22	27	29
3	R	30	24	R	31	23	R	16	20	26	27
4	R	31	25	R	31	21	28	29	19	25	28
5	R	32	26	R	30	22	27	27	20	24	28
6	R	30	25	R	31	20	27	28	19	29	26
7	R	33	28	R	30	20	25	34	18	24	26
8	R	32	25	R	31	21	29	30	19	24	30
9	R	35	28	R	28	20	30	29	19	25	30
10	R	34	25	R	31	25	28	29	22	28	28
11	R	31	29	R	29	20	28	27	19	23	21
12	R	30	27	18	32	21	28	31	20	25	30
13	R	30	24	R	31	20	27	34	18	24	26
14	R	34	25	R	33	20	29	29	24	23	25
15	R	30	25	18	34	23	23	31	21	26	30
16	R	31	26	18	32	20	29	34	20	23	30
17	R	30	26	R	30	22	28	35	21	25	29
18	R	31	24	19	34	22	31	28	21	26	26
19	R	30	25	R	31	24	35	32	21	24	27
20	R	30	28	R	30	19	26	29	22	27	28
21	R	29	25	R	29	23	26	35	20	25	30
22	R	36	24	R	29	23	31	33	19	24	29
23	18	33	25	22	33	22	33	35	20	25	35
24	R	33	24	R	30	25	31	22	24	29	28

No *P. aeruginosa* isolate showed resistance to any of the aminoglycosides (Table 4).

Adverse outcomes associated with the keratitis caused by these clinical strains may be attributed to the associations between virulence characteristics, which may function co-operatively. Further investigations are required to understand the mechanisms involved in *P. aeruginosa* virulence, which in effect provide the tools to

Table 4. Minimum inhibition concentration of antibiotics.

Isolate	Antibiotic											
	Norfloxacin	Aztreonam	Ceftazidime	Ciprofloxacin	Meropenem	Impienim	Gentamicin	Netilmicin	Amiacin	Tazobactam	Cefotaxime	Ceftriaxone
	N/A	N/A	N/A	≤ 0.5-1	≤ 1	≤ 1-4	≤ 1-4	≤ 2-8	≤ 4-8	≤8	8-32	>8-32
1	S	S	S	S	S	S	S	S	S	S	I	I
2	S	S	S	S	S	S	S	S	S	S	I	I
3	S	S	S	S	S	R	S	S	S	S	I	I
4	S	S	S	S	S	S	S	S	S	S	I	I
5	S	S	S	S	S	S	S	S	S	S	I	I
6	S	S	S	S	S	S	S	S	S	S	I	R
7	S	S	S	S	S	S	S	S	S	S	R	I
8	S	S	S	S	S	S	S	S	S	S	I	R
9	S	S	S	S	S	S	S	S	S	S	I	I
10	S	S	S	S	S	S	S	S	S	S	I	I
11	S	S	S	S	S	S	S	S	S	S	I	I
12	S	S	S	S	S	S	S	S	S	S	I	S
13	S	S	S	S	S	S	S	S	S	S	I	I
14	S	S	S	S	S	S	S	S	S	S	I	I
15	S	S	S	S	S	S	S	S	S	S	I	I
16	S	S	S	S	S	S	S	S	S	S	I	I
17	S	S	S	S	S	S	S	S	S	S	I	R
18	S	S	S	S	S	S	S	S	S	S	I	S
19	S	S	S	S	S	S	S	S	S	S	I	I
20	S	S	S	S	S	S	S	S	S	S	I	I
21	S	S	S	S	S	S	S	S	S	S	I	I
22	S	S	S	S	S	S	S	S	S	S	I	R
23	S	S	S	S	S	S	S	S	S	S	S	S
24	S	S	S	S	S	S	S	S	S	S	I	R

rapidly monitor newly virulent strains and provide better strategies to contain the disease.

DISCUSSION

There is a continuous increase in the use of contact lenses in Saudi Arabia because of the optical, occupational and cosmetic advantages to individuals. The unique structure of the human eye, the use of contact lenses and the constant exposure of the eye directly to the environment renders it vulnerable to a number of uncommon infectious diseases caused by microorganisms. Host defences directed against these pathogenic microorganisms, once anatomical barriers were breached, were usually inadequate to prevent loss of vision (Sankaridurg et al., 2000). Therefore, necessary precautions are required to protect the eye from these opportunistic organisms. These microorganisms and their pathogenic effects might be different from country to country, particularly in the developing countries (Jabs et al., 1995; Ragupathy et al., 2009). Therefore, the timely identification of the microorganisms found in contact lenses of Saudi wearers is of paramount importance.

Nevertheless, conditions may occur during lens wear such as microscopic trauma in the corneal epithelium, reduction in tear volume and as well reduction of aerobic normal epithelial metabolism may cause the bacterium to become opportunistic and cause infection (Mondino et al., 1986). It is therefore obvious that there are controversies about the effect of soft contact lenses on ocular microbiota and the associated diseases. Many authors reported that asymptomatic lens wear for extended periods did increase ocular microbiota (Larkin et al., 1991; Hart et al., 1993) and others reported that asymptomatic lens worn for extended periods did not

increase normal ocular microbiota (Gopinathan et al., 1997; Willcox et al., 1997). However, Efron et al. (2005) suggested that ocular diseases of contact lens wearer could be as a result of noncompliance or omission of surfactant cleaning rub and rinse steps, the use of disinfecting solution of marginal efficacy and lenses that attract and rapidly deposit protein. Thus, the lens care regimen is an important factor for consideration on subjects that showed no growth among the daily and extended contact lenses wearers.

However, due to the small sample size in each year, this trend was not statistically significant. It must be also noted that the present study and other studies referenced above are based on *in vitro* results, which do not necessarily mirror the clinical response to an antibiotic and could differ to the drug efficacy demonstrated *in vivo* (Kunimoto et al., 1999; Smitha et al*.,* 2005). Nevertheless, this *in vitro* study supports the concern about emerging fluoroquinolone-resistant *P. aeruginosa* strains in ocular infections, and highlights the need for continuous monitoring of emerging bacterial resistance. All isolates were susceptible to the fluoroquinolones (ciprofloxacin) in the MIC test.

Fluoroquinolones are commonly used as topical monotherapy for corneal infections. Since the introduction of second-generation fluoroquinolones ciprofloxacin and ofloxacin in the 1990s, the reported incidence of *in vitro* resistance to these antibiotics among bacteria isolated from bacterial keratitis and endophthalmitis has been steadily increased in the USA (Hwang, 2004) and India (Smitha et al*.,* 2005). In the current study, 11% of all keratitis isolates in Australia were non-susceptible to ofloxacin, which is higher than the results of previous report (Zhu et al*.,* 2006) in which the strains used were isolated through the years 1986 to 2004. An increasing trend of fluoroquinolone resistance was found in non-contact lens-related isolates, as the resistance rate increased from 8% (2/24) before year 2005 to 24% (4/17) from year 2006 (Choy et al., 2008).

Conclusion

The most common bacteria that contaminate contact lenses and its accessories were *S. epidermidis* and *P. aeruginosa*. The results suggest that *P. aeruginosa* isolated from different infectious samples may have different characteristics. We found that all strains of *P. aeruginosa* were resistant to the antibiotic Cefotaximee except one strain, while three strains were sensitive to Ceftriaxone; so these antibiotics still can be used in the treatment of infections caused by these sensitive strains of *P. aeruginosa*.

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