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Jake OO (2002). Pharmaceutical Interactions between *Striga hermonthica* (Del.) Benth. and fluorescent rhizosphere bacteria Of *Zea mays*, L. and *Sorghum bicolor* L. Moench for *Striga* suicidal germination In *Vigna unguiculata*. PhD dissertation, Tehran University, Iran.

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Full Length Research Paper

Seroprevalence of cytomegalovirus infection among pregnant women at Omdurman Maternity Hospital

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This study was conducted to determine the seroprevalence of human cytomegalovirus (HCMV) among pregnant women at Omdurman Maternity Hospital between the period January 2009 and June 2009. Descriptive cross-sectional study was conducted in Omdurman Maternity Hospital; blood was taken from pregnant women that came for delivery and investigated for cytomegalovirus specific immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies using enzyme-linked immunosorbent assay (ELISA). Demographic and clinical data were collected by questionnaire after a written consent. A total of 200 pregnant women were included in this study. The ages of all women tested ranged from 18 to 43 years. Out of the 200 pregnant women tested, 195 (97.5%) and 12 (6.0%) were CMV IgG and CMV IgM positive, respectively. The age was associated with CMV IgM and history of miscarriage was significantly associated with CMV IgG positive women, while parity, congenital abnormalities, educational level, and occupation were not significantly ($P > 0.05$) associated with CMV infection.

Key words: Seroprevalence, human cytomegalovirus (HCMV), pregnant women.

INTRODUCTION

The human cytomegalovirus (HCMV) or human herpes virus 5 is one of the major causes of congenital infections (Kenneson and Cannon, 2007; Dollard et al., 2007; Cannon, 2009; Munro et al., 2005; Sotoodeh et al., 2010). Its clinical manifestations range from asymptomatic forms (90% of cases) to severe fetal damage, and in rare cases, death due to miscarriage. Furthermore, 10 to 15% of the children who are asymptomatic at birth may develop late sequelae, especially hearing defects, after a period of months or even years (Massimo et al., 2009).

HCMV can be transmitted via saliva, sexual contact, placental transfer, breast feeding, blood transfusion and solid-organ transplantation (Bowden, 1991). Latency following a primary infection may be punctuated by periodic reactivations that give rise to recurrent infections,

and *in utero* transmission may occur during either primary or recurrent infections. Although the mechanisms and the pathogenesis of intrauterine transmission and severe fetal infection in the presence of preexisting maternal immunity are unknown, an analysis of CMV strain-specific antibody responses revealed an association between intrauterine transmission of CMV and reinfection with new or different virus strains in sero-immune women (Bappona, 2001), but it is likely that most recurrent infections are due to reinfection. The risk of congenital infection is much higher during primary infection (Fowler and Boppana, 2006). It has been reported that the risk of fetal damage is greater if the primary infection occurs during the first trimester of pregnancy (Adler and Marshall, 2007).

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CMV is a slow replicating virus from the herpes family, infecting only as many as 1% of all neonates in developed countries, but demonstrating up to 90% immunoglobulin G (IgG)-positivity in developing countries (Cannon, 2010). As well as increasing with age, CMV seroprevalence may also depend on sexual activity and occupation, particularly occupations involving close contacts with children in a community setting. In the case of parents, contact with the urine or saliva of their children is a major source of infection (Adler, 1991).

CMV is the most common and serious congenital infection, because it occurs after both primary and recurrent infection in pregnancy and is a major cause of childhood deafness and neurological handicap (Nigro, 2009, Colugnati et al., 2007).

High CMV seroprevalence (98.3%) among pregnant women was reported (Nahla et al., 2011). Previous study conducted at Omdurman Maternity Hospital revealed that the seroprevalence of CMV IgG antibodies among pregnant women was 95% (Kafi et al., 2009). A recent study conducted at El-Rahad hospital in Western Sudan reported that the seroprevalence of CMV among pregnant women was 72.2 and 2.5% for CMV IgG and CMV immunoglobulin M (IgM), respectively (Hamdan et al., 2011).

Various other unpublished observations emphasize the clinical importance of CMV infections among pregnant women and its detrimental consequences to their infants in Sudan; however, work on IgM and its association with congenital anomalies of neonates is very scarce, this study was intended to know the magnitude of this problem in Sudan and to know if there is a need for vaccination.

MATERIALS AND METHODS

This is a descriptive cross-sectional study conducted in Omdurman Maternity Hospital between January 2012 and June 2012. A total of 200 pregnant ladies attending the hospital were selected randomly from all women that came for delivery during the study period. Omdurman Maternity Hospital is the biggest maternity hospital in Khartoum. Khartoum is the capital of Sudan with an area of 28.140 km². The total population is about 6000000. It is divided into three provinces: Khartoum, Omdurman and Bahary. The hospital serves population of these provinces; area around Khartoum and in addition to referred patients from other states of Sudan, total deliveries was about 27000 in 2011. A total of two hundred venous blood samples were collected. The blood samples were collected under aseptic conditions, allowed to clot, centrifuged at 3000 rpm for 5 min, and sera were collected in sterile containers and stored at -40°C until tested. The Enzyme-Linked Immunosorbent Assay (ELISA) was used to detect the specific HCMV IgG and IgM antibodies. A well constructed questionnaire was filled by the investigator; a written consent was obtained from all the women that participated.

RESULTS

Overall prevalence of anti-CMV IgG antibodies in pregnant women attending Omdurman Maternity Hospital for

delivery was 97.5%. 195 women out of 200 women studied were positive for CMV IgG, while only 6% were CMV IgM positive. The results showed that the highest anti-CMV IgG seropositivity rate was among those with 40 years and more, while the lowest rate was among women less than 20 years old (Table 1).

CMV seropositivity was analyzed with respect to parity. No statistically significant difference was found between primigravidas and multiparous women on CMV infection (Table 2). Out of the 195 women who were CMV IgG positive, 8 women (4.2%) were reported on having child with congenital abnormalities, while no one from the negative group was reported on having congenitally formed child. In this study, no significant difference ($P > 0.05$) was found between working and non working women in CMV seropositivity (Table 3). The study demonstrates that the level of education of pregnant women had no effect on CMV seropositivity (Table 4).

DISCUSSION

This study revealed that the prevalence of CMV in pregnant women is very high, anti-CMV IgG antibodies was found in 97.5% of the cases, while 6% of the subjects tested positive for anti-CMV IgM.

The detection of CMV IgG indicated that the pregnant women had previously been infected with CMV. After CMV infection, IgG remains in the body for life and protects considerably against the next infections. This indicates that a negative results of CMV IgG test means that the women have not been infected with the virus.

The seroprevalence of CMV IgG observed in this study was similar to the results reported in Sudan by Nahla et al. (2011) which was 98.3% and Kafi et al. (2009) which was (95%). The picture of CMV prevalence in different countries is almost similar to our results; 96% in Egypt (El-Nawawy et al., 1996), 97.2% in Nigeria (Akinbami et al., 2011), 97.3% in Turkey (Uyar et al., 2008), 98.1% in Korea (Seo et al., 2009), and 95.6% in China (Meng et al., 2011). However, the results of this study were higher than those reported by Picone et al. (2009) in France (46.8%), Alanen et al. (2005) in Finland (56.3%), and Staras et al. (2006) in the United State (60.0%). It seems that the prevalence of CMV infection observed in this study was similar to that reported in other developing communities but higher than in the developed communities. This may be attributed to the low socioeconomic status and poor hygienic practices which might play important roles in increasing the rate of CMV infection. It was previously documented that seroprevalence of CMV among women varies with geographical location, socioeconomic status and occupation (Awosere et al., 1999).

In the present study, the rate of positive CMV IgM was 6.0% among tested pregnant women, which reflected an active recent infection or reactivation of the virus. This finding was higher than that of Hamdan et al. (2011) in Western Sudan who reported the rate of positive CMV

Table 1. Distribution of CMV seropositive women according to age group.

Age group (Years)	No. tested	Anti-CMV IgG positive		CMV IgM positive	
		No.	%	No.	%
>20	38	36	94.7*	2	5.2
20-29	78	76	97.4	4	5.1
30-39	74	73	98.6	3	4
≥ 40	10	10	100	3	30
Total	200	195	97.5	12	6

CMV: Cytomegalovirus.

Table 2. The effect of frequency of parities on CMV IgG and IgM seropositivity among pregnant women.

Serological marker (Anti-CMV antibodies)			Frequency of parities			
			1 time	2 times	3 times	< 3 times
Anti-CMV IgG	Positive	Frequency	62	38	21	74
		%	31.0	19.0	10.5	37.5
	Negative	Frequency	2	2	1	0
		%	1.0	1.0	0.5	0.0
Anti-CMV IgM	Positive	Frequency	2	3	3	4
		%	1.0	1.5	1.5	2.0
	Negative	Frequency	62	38	22	66
		%	31.0	19.0	11.0	32.0

CMV: Cytomegalovirus.

Table 3. Distribution of CMV seropositive women according to occupation, history of abortion and history of congenital anomaly baby.

Variable	CMV IgG positive	CMV IgG negative	Comment
Occupation			
Workers (%)	110 (56.4)	2 (40)	
Not workers (%)	85 (43.6)	3 (60)	
Congenital anomaly			
Yes (%)	8 (4.2)	0	
No (%)	187 (95.8)	5 (100)	
History of miscarriage			
Yes (%)	20 (10.2)	0	
No (%)	175 (89.8)	5 (100)	

IgM as 2.5%; however, far higher CMV IgM seroprevalence (94.3%) in neonates was recently reported in Sudan (Nahla et al., 2011). Variable IgM positivity were reported worldwide, only 1.0% in Turkey (Uyar et al., 2008), 2.5% in Iran (Bagheri et al., 2012) and 1.7% in Korea (Seo et al., 2009). However, the findings of this study were in agreement with that obtained by Saraswathy et al. (2011) in Malaysia (7.2%), but lower than that reported by Arabzadeh et al. (2005) in Iran (33

%) and Lone et al. (2004) in Kashmir valley (15.98%).

Our study showed a significant association ($P < 0.05$) between the history of miscarriage and CMV IgG seropositivity, and this may be due to infection by CMV earlier in reproductive live causing miscarriage.

In this study, a significant association ($P < 0.05$) was found between the age of pregnant women and CMV IgM seropositivity. Majority of CMV IgM positive women were above 30 years of age. This finding agreed with the

Table 4. Anti-CMV IgG seropositivity rate by educational levels.

Educational level	No. tested	Anti-CMV IgG positive	
		No.	%
Illiterate	18	18	100
Primary school	85	84	98.8
Intermediate school	44	43	97.7
Secondary school	47	46	97.8
High education	6	6	100
Total	200	195	-

results of Bate et al. (2010) in the United States. However, our finding disagreed with the results obtained by Hamdan et al. (2011) in Western Sudan, which could be attributed to the difference in the mean of age for tested women. According to this finding, pregnant woman above 30 years of age were at higher risk of CMV infection.

In this study, the parity, gestational age, congenital abnormalities, educational level, residence and occupation were not significantly ($P > 0.05$) associated with CMV infection among pregnant women. However, other authors reported significant association of these risk factors to CMV infection among pregnant women (Hamdan et al., 2011; Bagheri et al., 2012). The fact that there were no differences related to the age of the women indicates the same behavior at different ages.

The findings of our study indicated high prevalence of CMV seropositivity among pregnant women at Omdurman Maternity Hospital. Furthermore, the results showed that maternal age was a main risk factor for CMV reinfection or new infection. IgG avidity test should be used to distinguish primary and recurrent infection, and polymerase chain reaction (PCR) is essential for accurate diagnosis of CMV infection. CMV infection may play an important role in miscarriage. Introduction of national screening and immunization is a matter of discussion especially in areas with high prevalence of IgG and poor countries like Sudan.

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Full Length Research Paper

Towards the development of rapid biofilm antibiotic sensitivity testing (BAST)

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This study presents a modification of the antibiotic susceptibility testing (AST), which is a rapid means of determining the response of planktonic bacteria to different antimicrobial agents, for application to biofilms. Colony biofilm was first developed on a cellulose filter/membrane disc, over which an antibiotic disc was imposed. Zone of inhibition was measured after incubation on nutrient agar. Biofilms were not as susceptible to the test antibiotics as compared to the planktonic cultures. The results point to the possibility of this method as a rapid means for antibiotics for treating biofilm infections. Limitations and potential application for biofilm AST are discussed.

Key words: Biofilm, antibiotic susceptibility testing.

INTRODUCTION

The antibiotic susceptibility testing (AST) is a rapid means of determining the response of bacteria to different antimicrobial agents. AST utilizes the disc diffusion method, which is simple and widely used for sensitivity testing (Bauer et al., 1966; Clinical and Laboratory Standards Institute, 2009). Disc diffusion is very flexible, with a wide variety of antibiotic discs of varying concentrations available. This test effectively identifies organisms that are susceptible/resistant to certain antibiotics through visual means. Identification of effective antibiotics will lead to the successful treatment of bacterial infections. However, the test is conducted on planktonic cultures, which differ from biofilms in their response to antimicrobials *in vivo* (Jorgensen and Ferraro, 2009).

Studies show that 80% of infectious diseases are due to biofilm bacteria that are more difficult to treat than planktonic bacteria. (Lewis, 2005; Walters et al., 2003). Most biofilm antibiotic susceptibility tests utilized

polystyrene wells for growing biofilms *in vitro* (Cerca et al., 2005; Ceri et al., 1999; Olson et al., 2002; Amorena et al., 1999). Results are not easily visualized compared to the disc assay. Therefore, it is necessary to develop a rapid test that will identify antibiotics for effectively treating biofilm infections.

This study presents a modification of the antibiotic susceptibility testing performed on planktonic cultures for application to biofilm cultures.

MATERIALS AND METHODS

Preparation of bacterial culture

Overnight cultures of *Pseudomonas aeruginosa* PA01 and *Staphylococcus aureus* SH1000 were prepared by dispersing a single colony in 5-ml test tubes in Mueller-Hinton broth (MHB). After incubation at 37°C in air with shaking, 500 µl of the overnight cultures were transferred to a 50 ml flask with 10 ml MHB, and

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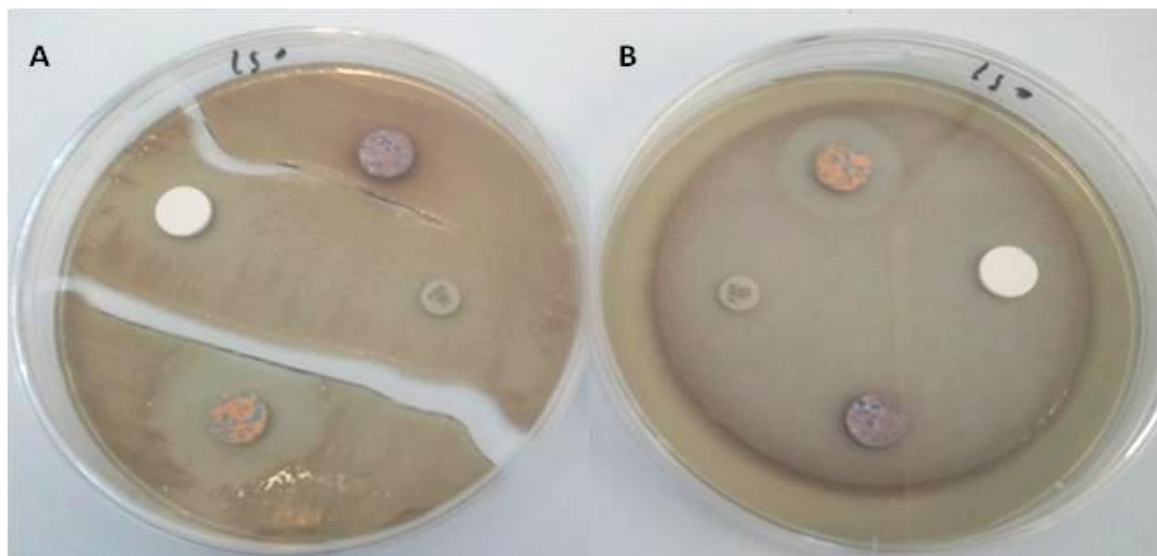


Figure 1. Comparison of the results of the antibiotic sensitivity assay for (A) planktonic culture, and (B) biofilm of *Pseudomonas aeruginosa* PA01. Antibiotic discs: white, chloramphenicol; orange, imipenem; small greyish, cefoxitin; and blue, vancomycin.

incubated with shaking at 120 rpm at 37°C. After 4 h, the bacterial cultures were diluted to an optical density (OD) of 0.05 at 600 nm.

Initial antibiotic sensitivity tests

For the AST, 1 ml of the culture was added to 10 ml of lukewarm broth, and poured over brain heart infusion (BHI) agar plates for *S. aureus* SH1000 and Iso-Sensitest™ Agar plates for *P. aeruginosa* PA0. Four antibiotic discs, with the following concentration were arranged over the agar: 30 µg chloramphenicol, 30 µg cefoxitin, 30 µg vancomycin, and 10 µg imipenem. The plates were incubated at 37°C in air for 48 h, after which the zone of inhibition was measured.

To perform the biofilm AST (BAST), cellulose ester discs or filters with 0.22 µm pore size and 7 mm diameter were sterilized by autoclaving for 15 min at 121°C. After cooling, the discs were soaked overnight in phosphate buffered saline. These were then dipped into overnight bacterial cultures that have been diluted to an OD 0.05 at 600 nm. The filters were placed carefully on the surface of BHI agar for *S. aureus* and Iso-Sensitest agar for *P. aeruginosa*, followed by incubation at 37°C for 48 h. Discs were then carefully removed and dipped in 4% human plasma in order to promote the adherence of bacteria to the membranes. After dipping, the membranes were placed onto new plates with BHI and Iso-Sensitest media. The same antibiotic discs used in the AST were then superimposed over the membrane filters and the zone of inhibition of bacterial growth was measured after 48 h.

Verification tests with strain-specific antibiotics

Using the same protocol as mentioned earlier, a second antibiotic sensitivity test was conducted; this time the antibiotics used were specific for the bacterial strains. The following antibiotics were tested on *S. aureus*: erythromycin (15 µg), ampicillin (10 µg), vancomycin (30 µg), gentamicin (10 µg) and cefotaxime (30 µg). For *P. aeruginosa*, the following antibiotic discs and concentrations were utilized: amikacin (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), imipenem (10 µg), and chloramphenicol (30 µg).

RESULTS AND DISCUSSION

The preliminary tests show the zones of inhibition of bacterial growth produced by the antibiotics (Figures 1 and 2). It is clear that the planktonic cultures were all sensitive to the 4 test antibiotics as compared to the biofilms. Figure 1 shows that it is only imipenem that was able to inhibit growth of the biofilm. *S. aureus* planktonic cultures exhibited overlapping zones while the growth zones in the biofilm were distinctly separated.

Table 1 summarizes the inhibitory action of the different antibiotics on the bacterial cultures of the two strains tested. In the preliminary trial, planktonic cultures of *S. aureus* were most sensitive to the four test antibiotics. *S. aureus* biofilm was sensitive to the action of three of the four antibiotics tested. However, the zones of inhibition were smaller than those observed for the planktonic cultures, which implies that the biofilms were able to grow despite the antibiotics. Disc zones were also observed around the antibiotics imipenem and chloramphenicol in planktonic cultures of *P. aeruginosa*. Imipenem was the only antibiotic that produced a zone of inhibition on the *P. aeruginosa* biofilm.

After the initial tests, strain-specific antibiotics were used. Planktonic *S. aureus* cultures differed in their response to the five test antibiotics. Its growth was highly inhibited by cefotaxime and ampicillin, while least inhibited by vancomycin. Compared to planktonic cultures, the biofilms were generally less affected by the antibiotics. Cefotaxime, which was highly inhibitory to planktonic growth, did not produce growth inhibition against the biofilm. The same was observed for vancomycin-treated biofilm. Erythromycin, ampicillin, and gentamicin all inhibited biofilm growth, but not as effectively as compared to

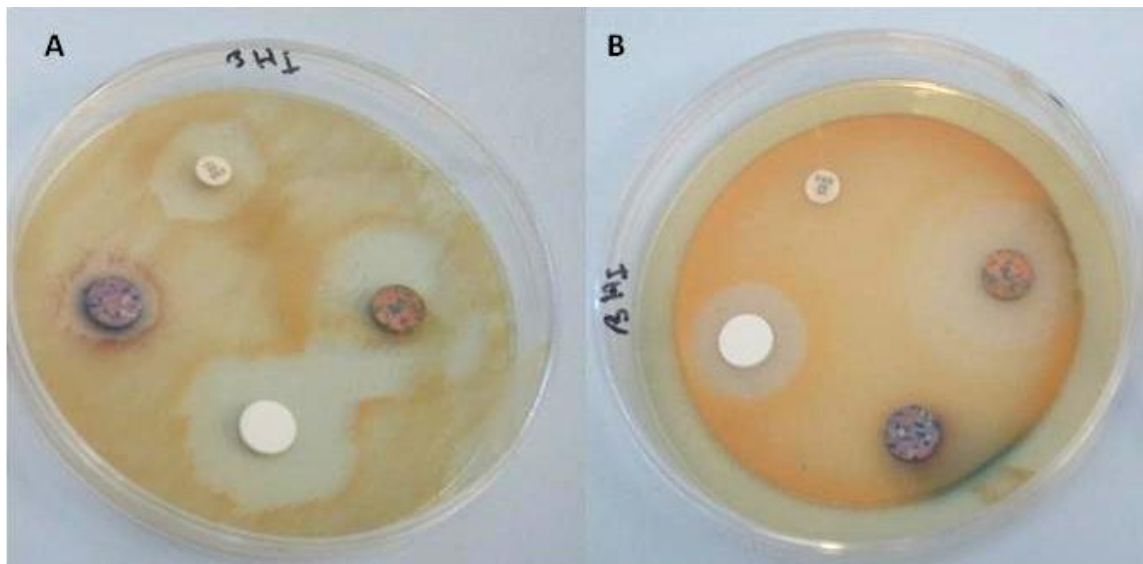


Figure 2. Comparison of the results of the antibiotic sensitivity assay for (A) planktonic culture, and (B) biofilm of *Staphylococcus aureus* SH1000. Antibiotic discs: white, chloramphenicol; orange, imipenem; small greyish, cefoxitin; and blue, vancomycin.

Table 1. The zone of inhibition of specific antibiotics on the growth of planktonic and biofilm cultures of *S. aureus* and *P. aeruginosa*.

Antibiotic	Zone of inhibition (mm \pm SD)				
	<i>S. aureus</i> SH1000		<i>P. aeruginosa</i> PA01		
	Planktonic	Biofilm	Planktonic	Biofilm	
Initial test	Cefoxitin	15 \pm 0.5	0	0	0
	Imipenem	25 \pm 1.0	20 \pm 2.5	13 \pm 1.5	9 \pm 0.6
	Vancomycin	10 \pm 2.0	5 \pm 1.2	0	0
	Chloramphenicol	25 \pm 1.0	15 \pm 1.2	12 \pm 1.15	0
Verification test	Erythromycin	15 \pm 1.0	8 \pm 1.0	Not tested	Not tested
	Ampicillin	25 \pm 1.5	8 \pm 2.0	Not tested	Not tested
	Vancomycin	8 \pm 0.6	0 \pm 0	Not tested	Not tested
	Gentamicin	15 \pm 1.5	10 \pm 2.0	Not tested	Not tested
	Cefotaxime	23 \pm 1.0	0 \pm 0	Not tested	Not tested
	Amikacin	Not tested	Not tested	20 \pm 1.7	6 \pm 0.6
	Ceftazidime	Not tested	Not tested	20 \pm 0.6	6 \pm 1.5
	Ciprofloxacin	Not tested	Not tested	30 \pm 2.5	10 \pm 2.0
	Imipenem	Not tested	Not tested	25 \pm 1.2	0 \pm 0
	Chloramphenicol	Not tested	Not tested	0 \pm 0	0 \pm 0.6

*Values are means of 3 replicates. SD: Standard deviation.

their inhibition of planktonic cultures. Except for gentamicin, growth of biofilms was more than 50% higher in the presence of erythromycin and ampicillin; gentamicin proved to be most inhibitory of the antibiotics to the *S. aureus* biofilm.

In the planktonic culture of *P. aeruginosa*, four of the five test antibiotics were able to produce zones of growth

inhibition ranging from 20 to 30 mm (Table 1). Based on the zone of inhibitions produced, the growth of biofilms was 70% more than planktonic growth despite the addition of amikacin, ceftazidime, and ciprofloxacin. In the case of imipenem, it was highly effective against planktonic cells, but not against the biofilm. This last result was not in agreement with results of the initial tests

where imipenem inhibited biofilm growth of *P. aeruginosa*. Chloramphenicol was ineffective against *P. aeruginosa* because there was no zone of inhibition produced in both planktonic cells and biofilms.

The presence of some conflicting results indicates that more precision is necessary in carrying out the antibiotic tests. As in the case of the antibiotic sensitivity test for planktonic cultures, strict quality standards have to be met before BAST is performed. Extreme care is necessary during the preparation of the materials (that is, culture media, pH, and antibiotic discs) for the test. In addition, the preparation of biofilms on membranes has to be perfected to ensure that growth is homogeneous and there are no contaminating strains. Although such a condition is ideal; since natural biofilms are heterogeneous (Boles et al., 2004), a single strain biofilm could prove sufficient for antibiotic sensitivity testing. The interpretation of the antibiotic disc inhibition zones has to follow standards in order to attain reliable results and to give proper recommendation. Therefore, the standards for the performance and the interpretation of BAST results also have to be developed.

Conclusion

The proposed BAST method appears to be effective in identifying antibiotics for treating biofilm infection. These preliminary results show that there is a potential of developing BAST as a sensitive assay for biofilms. More studies should be conducted for producing biofilms *in vitro*, standardizing methods, reagents, and conditions for BAST.

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UPCOMING CONFERENCES

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13th Congress of the Asia-Pacific Federation for Clinical Biochemistry and Laboratory Medicine (APCCB 2013), Bali, Indonesia, 27 Oct 2013



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