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ANTIMICROBIAL ACTIVITIES OF AQUEOUS LYSATE OF *ACANTHAMOEBA* SPP AGAINST SELECTED PATHOGENIC BACTERIA

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ABSTRACT

The increasing of infectious diseases and emerging of multi-drug resistant bacteria are worldwide problems that require a search for a new potential drug from various sources including free-living amoebae to overcome these problems. In this study, the aqueous lysates of two isolates of *Acanthamoeba* viz *A. castellanii,* a clinical isolate designated as IMR isolate, and *Acanthamoeba* sp., an environmental isolate designated as SW isolate were tested against two pathogenic bacteria, methicilin resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus aureus.* The anti-bacterial test was done using disc diffusion (Kirby Bauer) method to determine the minimum inhibition concentration (MIC) values of the lysates against the bacteria. Twofold dilution series of the *Acanthamoeba* lysates with concentrations ranging from 0.3 mg/mL to 2.4 mg/mL were used. The measurements of diameter of inhibition zone of the amoeba lysates against each bacterium were compared with the inhibition zone caused by a positive control (chloramphenicol) to analyse the effectiveness of the lysates as anti-bacterial agents. MRSA and *S. aureus* tested were slightly sensitive to both lysates of *Acanthamoeba* used in this study to suggest the potential of these lysates as bacterial agents. The data obtained were further confirmed by analysis of bacterial morphology under SEM.

Key words: *Acanthamoeba* lysates, SEM, MRSA, *Staphylococcus aureus*, MIC

ABSTRAK

Pertambahan penyakit berjangkit dan kemunculan bacteria rintan terhadap berbagai ubatan merupakan permasalahan di seluruh dunia yang memerlukan pencarian pelbagai sumber baharu sebagai ubatan termasuk ameba hidup bebas untuk mengatasi masalah ini. Dalam kajian ini, lisat akueus dari dua pemencilan *Acanthamoeba* iaitu *A. castellanii*, pemencilan klinikal ditetapkan sebagai isolat IMR dan *Acanthamoeba* sp., pemencilan dari sekitaran, ditetapkan sebagai isolat SW telah diuji terhadap dua bakteria pathogen yakni *Staphylococcus aureus* (MRSA) and *Staphylococcus aureus.* Ujian anti-bakteria dilakukan dengan menggunakan kaedah resapan cakra (Kirby Bauer) untuk menentukan nilai kepekatan perencatan minimum (MIC) lisat terhadap bakteria. Siri kepekatan duakali ganda lisat ameba dengan kepekatan berjulat 0.3mg/mL hingga 2.4 mg/mL digunakan. Pengukuran diameter kawasaan perencatan oleh lisat ameba ke atas setiap bakteria dibanding dengan kawasan perencatan oleh kawalan positif (chloramphenicol) untuk menganalisis keberkesanan lisat sebagai agen anti-bakteria. MRSA dan *S. aureus* yang diuji didapati sensitif terhadap kedua-dua lisat *Acanthamoeba* yang digunakan membuktikan keupayaannya sebagai agen anti-bakteria. Data yang diperolehi disahkan dengan melakukan analisis morfologi bakteria di bawah SEM.

Kata kekunci: *Acanthamoeba* lysates, SEM, MRSA, *Staphylococcus aureus*, MIC

INTRODUCTION

Antimicrobials are natural or synthetic drugs which inhibit or kill the bacteria so they are used to control of deadly infectious diseases caused by a large variety of pathogenic bacteria. Today, more than 15 different classes of antimicrobials are known which differ in their chemical structure and mechanisms of action (WHO, 2003). Meanwhile, antimicrobial resistance among the bacteria continues to grow in alarming rate in distinct geographic regions, worldwide. New drug-resistant strains of pathogenic bacteria have emerged and proliferated in some areas of the world. For example, vancomycin-resistant

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enterococci have been reported in the the United States (Klugman & Feldman, 1999). And also most countries report significantly high rates of methicilin resistant *Staphylococcus* spp. in their medical centers (Jones, 1996). Infections due to these bacteria are often difficult to treat because of its virulence and a relatively limited choice of effective antimicrobial agents (Okuma *et al.*, 2002). In this study, two gram positive pathogenic bacteria (*Staphylococcus aureus and* Methicilin Resistant *Staphylococcus aureus)* were used to investigate the anti-bacterial activity of the amoeba lysates. Two isolates of *Acanthamoeba* from the environment and a clinical sample were used as sources for anti-bacterial agents in this study.

MATERIALS AND METHODS

Bacteria types and Cultivation

Two gram positive pathogenic bacteria used in this study were Methicilin Resistant *S.aureus* (MRSA) and *S. aureus*. These bacteria were originally obtained from Hospital Sultanah Nur Zahirah, Kuala Terengganu, Terengganu, Malaysia and were maintained in nutrient slant agar at 37ºC and kept in the Microbiology Laboratory, Faculty of Science and Technology, Universiti Malaysia Terengganu, Malaysia before use.

Acanthamoeba cultivation and lysate preparation

The isolates of *Acanthamoeba* viz a pathogenic *A. castellanii,* (IMR isolate) and an environmental isolate, *Acanthamoeba* sp (SW isolate) were grown in polypeptone liquid media at 30ºC. To obtain the amoeba lysates, the trophozoites of *Acanthamoeba* were harvested at the logarithmic growth phase by centrifugation (at 3000 rpm for 15 min). The cell pellets formed were washed twice with PBS and then re-suspended in 1000 mL PBS before 10 µL of protein inhibitor was added to the suspension. The pellet suspensions were sonicated (6 cycle, 30 sec each) before centrifugation at 20,000 rpm for 10 min. The supernatant parts (thus were called the aqueous lysates in this study) were used as the sources of the anti-bacterial agent in *Acanthamoeba.*

Anti-bacterial Test and Determination of Minimum Inhibitory Concentration (MIC)

Spread plate technique following the standard procedure was used to test the anti-bacterial activity of the *Acanthamoeba* lysates against the two bacteria used. About 0.5 mL of bacterial inoculums was spread evenly onto a sterile Petri disc containing solidified Mueller Hinton agar (MHA). Next, the antibacterial discs that were impregnated with different concentration of the *Acanthamoeba* lysates ranging from 0.3 mg/mL to 2.4 mg/mL were placed onto the surface of the Mueller- Hinton agar. Disc containing chloramphenicol was used as a positive control whereas disc containing Phosphate Buffer Saline (PBS) was used as a negative control in this test. The susceptibility of the bacteria to each *Acanthamoeba* lysate was determined by the formation of circular inhibition zones around the antibacterial discs after overnight incubation. This test allows the determination of minimum inhibitory concentration values (MIC) of the amoeba lysates against each bacteria tested.

Morphological observation under Scanning Electron Microscopy (SEM)

Acanthamoeba lysates that showed inhibition on the bacteria were used to examine the morphological changes of the bacteria under scanning electron microscopy (SEM). The IC_{50} values of the lysate were used to treat the bacteria and the bacteria were processed for SEM following established method.

RESULTS & DISCUSSIONS

Although not as strong as chloramphenicol, the results obtained show that both lysates of *Acanthamoeba* inhibited the growth of both gram positive bacteria used in this study (Table 1). Lysates of *A. castellanii* (IMR isolate) and *Acanthamoeba* sp (SW isolate) have almost equal strength of antimicrobial properties as shown by their MIC values (both at 1.2 mg/mL) although the diameter of the inhibition zone exhibited by lysate of *Acanthamoeba* sp. (SW isolate) was slightly bigger (7.0 mm, observed against MRSA) compared with the other lysate of *Acanthamoeba* (the diameter is 6.8 mm against MRSA and *S. aureus*).

The strength of these lysates to act as anti-bacterial agents compared with chloramphenicol (a positive control) against *S. aureus* and MRSA was approximately 35%. This percentage is considered good because the lysates of the *Acanthamoeba* spp. used were in the crude form. If bioactive compounds in these lysates can be purified and isolated, their antibacterial activities would be different, perhaps their antibacterial strength is comparable to chloramphenicol. Therefore, further study to use purified lysates with known bioactive compounds to reveal more of their potential as antibacterial-agent is strongly recommended.

Both lysates of *Acanthamoeba* possess anti-bacterial activity against the Methicilin Resistant *Staphylococus aureus* (MRSA) and *Staphylococus aureus.* These lysates however, do not have activity against *Pseudomonas aeruginosa,* a gram negative

Table 1. Measurement of diameter of inhibition zone (mm) of bacteria after treated with various concentrations of *Acanthamoeba* lysates. Values presented are mean±standard deviation from three replicates of data

Concentration of Lysates (mg/mL)	Amoeba lysates			
	A. castellanii		*Acanthamoeba* sp	
	S. aureus	MRSA	*S. aureus*	MRSA
Chloramphenicol	20.1±0	20.2±0.4	20.1±0.9	20.1±0.4
PBS	–	–	–	–
2.4	7.1±0.1 (35%)	7.1±0.6 (35%)	7.1±0.1 (35%)	7.1±0.1 (35%)
1.2	#6.8±0.1 (34%)	#6.8±0.5 (34%)	#6.8±0.5 (34%)	#7.0±0.2 (34%)
0.6	–	–	–	–
0.3	–	–	–	–

Note: - no inhibition zone, # minimum inhibition observed, Numbers in bracket are percentage activity compared with a positive control

bacteria (data not shown). This is probably mainly due to a significant different in the cell wall structure between the gram negative and the gram positive bacteria. The significance of cell wall type is very important to the survivability of the bacteria in different environments (Madigan & Martinko, 2005). This outer membrane protects the bacteria from several antibiotics or anti-bacterial agents which would normally damage the inner membrane or the peptidoglycan of the cell wall (Salton & Kim, 1996).

The antibacterial activity in lysates of *Acanthamoeba* against the MRSA and *S. aureus* as observed in the present study is probably similar to the activity of known antibiotics. Antibiotics stop or interfere with a number of cellular processes that bacteria rely on for growth and survival such as crippling production of the bacterial cell wall that protects the cell from the external environment. These antibiotics also interfere with protein synthesis by binding to the machinery that builds proteins from amino acids. They also cause disturbance in the cell metabolic processes, such as the synthesis of folic acid and block synthesis of DNA and RNA (Anderson, 2006). Other antibiotic such as penicillin targets the bacteria cell wall by disrupting the formation of the peptidoglycan layer. When this happens, the bacterium dies or hampered instead of reproducing (Kenneth, 2009). As a result, this kind of antibiotics is effective against Gram positive bacteria which have thick peptidoglycan layer. Thus, the *Acanthamoeba* lysates probably acted in similar manner as penicillin when treated against gram positive Methicilin Resistant *S. aureus* (MRSA) and *S. aureus*.

The underlying molecular mechanisms leading to antibiotic resistance can vary. Intrinsic resistance may naturally occur as a result of the bacteria's genetic makeup (Alekshun & Levy 2007). The bacterial chromosome may fail to encode a protein that becomes the target of the antibiotics. Acquired resistance in bacteria could have resulted from a mutation in the bacterial chromosome or the acquisition of extra-chromosomal DNA. The spread of antibiotic resistance mechanisms in bacteria occurs through vertical transmission of inherited mutations from previous generations and genetic recombination of DNA by horizontal genetic exchange (Witte, 2004).

Further analysis of anti-bacterial properties of the amoeba lysates involved the examination of the bacterial morphology under Scanning Electron Microscopy (SEM) after 24 h exposure to the lysates and the results are shown in Fig. 1. Morphological damaged and smaller in size with wrinkle appearances are apparent in bacteria treated with the amoeba lysates in contrast to the untreated cells which are round with smooth outer layer. Bigger diameter of inhibition zone by chloramphenicol in disc diffusion method to indicate its strong antibacterial property, was confirmed by SEM analysis. Morphology of all bacterial cells was severely affected after treated with this antibiotic (Fig 1). The changes in the cell (wall) morphology might affect the biological process of the bacteria, thus affect their growth and activity that can be translated as a clear zone of inhibition in disc diffusion assay.

The small zone of inhibition by the amoeba lysates in disc diffusion assay was due to the survivability of the bacteria after exposure to these lysates. Under SEM, not all cells of *S. aureus* and MRSA were observed to be affected by both lysates of *Acanthamoeba.* As a result, the non-affected cells are able to survive and resume their normal biological processes such as cell division and multiplication to increase their number. Therefore, only a small inhibition zone was exerted by these

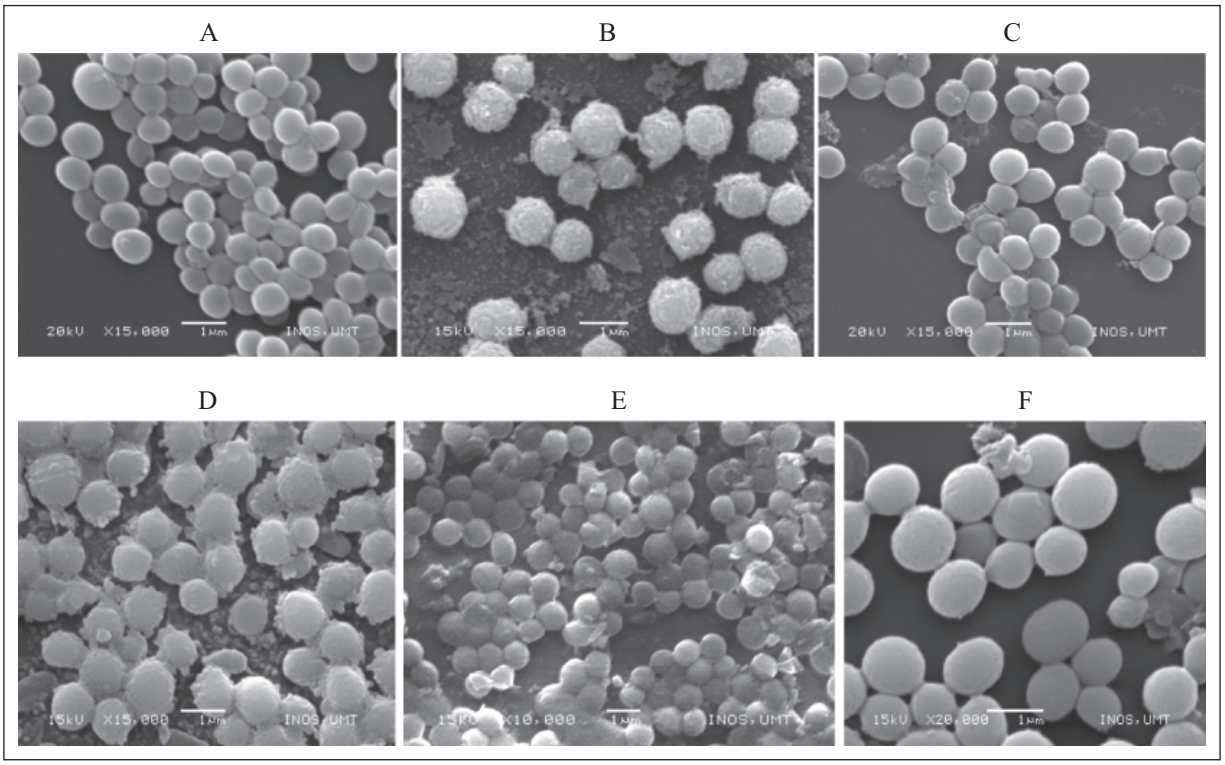

Fig. 1. SEM of MRSA and *S. aureus* after exposure to the *Acanthamoeba* lysates. Explanation for panels: A. MRSA when exposed to PBS, B. MRSA when exposed to chloramphenicol, C. MRSA when exposed to lysate of *A. castellanii* (IMR isolate), D. *S.aureus* when treated with Chloramphenicol, E. *S. aureus* when treated with lysate of *A. castellanii* (IMR isolate), F. *S. aureus* when treated with lysate of *Acanthamoeba* sp (SW isolate)

lysates as compared with the inhibition zone by chloramphenicol (positive control) after 24h incubation.

From this study, the lysates of *Acanthamoeba* had a promising effect on pathogenic bacteria such as MRSA and *S. aureus.* Lynch *et al.* (1982) reported that *E. histolytica*, the protozoan parasite causing amoebiasis, is capable of destroying the tissue of the infected host by producing a protein capable of forming pores in artificial lipid bilayers and target-cell membranes. This protein has a molecular mass of 28-30 kDa in its native state and of 13-15 kDa under denaturing and reducing conditions. The protein, named amoebapore, was partially purified from the 150,000 g supernatant of amoeba lysates (Rosenberg *et al.*, 1989). Studies on the effect of amoeba lysates or partially purified material on planar lipid bilayers suggested that oligomerization of active protomers occurred during formation of membrane pores (Keller *et al.*, 1989). Thus, similar proteins might also present in lysates of *Acanthamoeba* which have anti-microbial activity as observed in the present study.

In this study, all aqueous lysates of *Acanthamoeba* regardless of their pathogenecity background possess almost equal strength of antimicrobial properties. Interestingly, Nakisah *et al.* (2005) reported that lysate of a clinical isolate of *Acanthamoeba* is more potent as an anti-cancer agent compared to the lysate of non-pathogenic isolate. Pathogenic *Acanthamoeba* contains higher protease activities than non pathogens, which involved in the degradation of host tissues and major determinant of amoeba pathogenesis (Lorenzo-Morales *et al.*, 2005). In addition, Na *et al.* (2001) demonstrated that lysate of pathogenic *A. castellanii* causes severe damaged to HeLa cells compared with lysates of free living *Acanthamoeba*. Therefore, this study suggests that lysates of *Acanthamoeba* display slightly different anti-functional activity towards cancer cells lines and bacteria.

CONCLUSIONS

Aqueous lysates of both *Acanthamoeba* isolates used in this study have anti-bacterial activities against Methilin resistant *S. aureus* (MRSA) and *S. aureus*. The strength of their anti-bacterial activity (at their MIC values) is about 34% of activity exerted by chloramphenicol, a positive control used in this study. These results suggest the potential of these *Acanthamoeba* lysates as future anti-bacterial agents.

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