

Full Length Research Paper

Antimicrobial substances produced by bacteria isolated from different Jordanian sources that are active against methicillin-resistant *Staphylococcus aureus*

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Accepted 22 June, 2007

We report here the preliminary antimicrobial activity of substances produced by *Bacillus subtilis* NB-6 (air flora isolate), *Bacillus megaterium* NB-3 (air flora isolate), *Burkholderia mallei* NB-8 (water isolate) and *Corynebacterium kutscheri* NB-1 (soil isolate) against a number of methicillin-resistant *Staphylococcus aureus* (MRSA). The MRSA were isolated from sheep, bovine, camel and poultry meat samples collected from retail shops and slaughter houses located in Amman area, Jordan. *B. mallei* NB-8 and *C. kutscheri* NB-1 were found to possess a good antimicrobial activity against MRSA strains.

Key words: Antimicrobial activity, *Bacillus*, *Burkholderia*, *Corynebacterium*, methicillin-resistant *Staphylococcus aureus*.

INTRODUCTION

The spread of resistance to antibiotics undermines the therapeutic utility of anti-infective drugs in current clinical use (Bax et al., 2000). For example, *Staphylococcus aureus*, a major cause of community and hospital-acquired infections, has developed resistance to most classes of antibiotics, and isolates exhibiting such resistance is drawing great concern. Methicillin-resistant *S. aureus* (MRSA) strains appeared in the hospital environment after introduction of the semisynthetic penicillin, methicillin, leaving vancomycin as the last line of defense for MRSA treatment (Enright, 2003). With the appearance of vancomycin-resistant clinical isolates (Sieradzki et al., 1999), no antibiotic class is effective against multiresistant *S. aureus* infections. Thus, new antibiotic and therapy options are urgently needed to improve the management of bacterial infections (Saimann et al., 2001), and a major challenge is to find drugs that act against Methicillin-resistant *S. aureus*

(MRSA).

In this study, we report the discovery and preliminary antimicrobial activity of substances produced by *Bacillus subtilis* NB-6 (air flora isolate), *Bacillus megaterium* NB-3 (air flora isolate), *Burkholderia mallei* NB-8 (water isolate) and *Corynebacterium kutscheri* NB-1 (soil isolate) against methicillin-resistant *S. aureus* (MRSA) isolated from meat samples in Amman area, Jordan.

MATERIALS AND METHODS

Strains and media

Methicillin-resistant *S. aureus* strains (MRSA strain 1, 2, 3, 5, 6, 7, 8 and 13) were isolated from meat samples from sheep, bovine, camel and poultry in Amman area, Jordan (Quddoumi et al., 2006). The MRSA strains were deposited in Jordan Culture Collection (JOCC). The antimicrobial substances-producing bacteria, *B. subtilis* NB-6 (air flora isolate) (El-Banna, 2003), *B. megaterium* NB-3 (air flora isolate) (El-Banna, 2003), *B. mallei* NB-8 (water isolate) (El-Banna, 2005a) and *C. kutscheri* NB-1 (soil isolate) (El-Banna, 2005b), were isolated from different sources in Jordan and deposited in Jerash Culture Collection (JCC). MRSA strains were cultured at 37°C on mannitol salt agar (Difco). The antimicrobial substances-producing bacteria were cultured at 27°C on nutrient agar (Oxoid).

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Table 1. Antimicrobial activity of substances produced by Jordanian isolates (*Corynebacterium kutscheri* NB-1, *Bacillus megaterium* NB-3, *Bacillus subtilis* NB-6 and *Burkholderia mallei* NB-8) against methicillin-resistant *Staphylococcus aureus* (MRSA).

MRSA strains	Antimicrobial activity of strain ^a			
	NB-1	NB-3	NB-6	NB-8
MRSA 1	n.a. ^b	n.a.	n.a.	w.a.
MRSA 2	9.0	n.a.	n.a.	9.0
MRSA 3	n.a.	n.a.	n.a.	13.5
MRSA 5	12.9	n.a.	n.a.	14
MRSA 6	n.a.	n.a.	n.a.	n.a.
MRSA 7	n.a.	n.a.	n.a.	w.a.
MRSA 8	8.5	n.a.	n.a.	w.a.
MRSA 13	n.a.	w.a. ^c	n.a.	11

^aDiameter of inhibition zone (mm).

^bn.a.: No activity.

^cw.a.: Weak activity.

Preculture conditions

Bacteria were transferred by loop from slants to 125 ml flasks containing 50 ml medium like that used in subsequent experimental cultures. All precultures were incubated at appropriate temperature on a rotary shaker (Sanyo Gallenhamp PLC, Leicester, LE 3 2uz, UK) at 180 rpm for 24 h. 1 ml of preculture was used to inoculate all experimental cultures.

Agar diffusion test

Extraction of the active substance from cells grown in liquid cultures (100 ml) was carried out as follows. Briefly, cells were pelleted by centrifugation and extracted with acetone, and the supernatant was extracted with ethylacetate. Both extracts were evaporated by a rotary evaporator (Heidolph instruments, GmbH and Co KG Vertrieb, Kelheim, Germany) at < 50°C, and the dry substances (separately) were dissolved in 0.5 ml methanol. The antimicrobial activity of these extracts was carried against methicillin-resistant *S. aureus* strains (MRSA) by agar diffusion test. Filter discs containing 10 µl of the active substance dissolved in methanol (acetone extract or ethylacetate extract) were placed on the test plates. The plates were incubated at 37°C for 48 h, and the antimicrobial activity was determined by measuring zones of growth inhibition (El-Banna and Winkelmann, 1998).

Biotest plates preparation

Cell suspension of 24 h MRSA precultures were prepared (O.D₅₇₈ = 1), and 0.5 ml of this suspension was used to inoculate 250 ml agar medium (20 ml per plate, Arab food and Media Applicances Company limited, Zarka industrial area, Jordan).

RESULTS AND DISCUSSION

The agar diffusion method was used to compare the antimicrobial activity of *B. subtilis* NB-6, *B. megaterium* NB-3, *B. mallei* NB-8 and *C. kutscheri* NB-1 against

methicillin-resistant *S. aureus* (MRSA) isolated from meat samples from sheep, bovine, camel and poultry in Amman area, Jordan. As shown in Table 1, substances produced by *B. mallei* NB-8 showed good antimicrobial activity against MRSA 2, MRSA 3, MRSA 5 and MRSA 13, and weak antimicrobial activity against MRSA 1, MRSA 7 and MRSA 8, while no antimicrobial activity against MRSA 6. MRSA 2, MRSA 5 and MRSA 8 were inhibited by substances produced by *C. kutscheri* NB-1. The other MRSA strains (MRSA 1, 3, 6, 7 and 13) were not inhibited at all. All MRSA strains were not inhibited by substances produced by *B. subtilis* NB-6 and *B. megaterium* NB-3 except MRSA strain 13 which was slightly inhibited by *B. megaterium* NB-3 produced substances.

S. aureus is an important cause of a variety of diseases in human and animals worldwide (Gilot and Leeuwen, 2004). Their ability to cause diseases is associated with several pathogenic factors including extracellular enzymes and toxins (Lee, 2003). Both animal and human isolates are generally resistant to penicillins (Seguin et al., 1999). Several reports suggest that the transfer of *S. aureus* between human and cattle is possible and that the infection of human by transmission through food products contaminated with animal MRSA is very plausible (Lee, 2003; Kaszanyitzky et al., 2003). Romero-Tabarez et al. (2006) has previously reported an antimicrobial activity of substances produced by *B. subtilis* (soil isolate) against multidrug-resistant bacterial pathogen including methicillin-resistant *S. aureus*.

In this study, we have described the antimicrobial activity of substances produced by bacteria against several methicillin-resistant *S. aureus* strains which may be promising for the development of new drugs against microbial pathogens.

REFERENCES

- Bax R, Multan N, Verhoef (2000). The millennium bugs-the need for and development of new antibacterials. *Int. J. Antimicrob. Agents*. 16: 51-59.
- El-Banna N (2003). Antimicrobial substances produced by air flora. *Arab Gulf J. Sci. Res.* 21: 134-139.
- El-Banna N (2005a). Antimicrobial activity of *Burkholderia mallei* isolated from Jordanian water pond. *Umm Al-Qura. Univ. J. Sci. Med. Eng.* 17: 31-40.
- El-Banna N (2005b). A study on the antimicrobial activity of *Corynebacterium kutscheri* isolated from Jordanian soil. *Umm Al-Qura. Univ. J. Sci. Med. Eng.* 17: 1319-8904.
- El-Banna N, Winkelmann G (1998). Pyrrolnitrin from *Burkholderia cepacia*: antibiotic activity against fungi and novel activities against streptomycetes. *J. Appl. Microbiol.* 85: 69-76.
- Enright M (2003). The evolution of resistant pathogens-the case of MRSA. *Curr. Opin. Pharmacol.* 3: 474-479.
- Gilot P, Leeuwen W (2004). Comparative analysis of *agr* locus diversification and overall genetic variability among bovine and human *Staphylococcus aureus* isolates. *J. Clin. Microbiol.* 42: 1265-1269.
- Kaszanyitzky E, Jonosi S, Egyd Z, Agost G, Semjen G (2003). Antibiotic resistance of staphylococci from human, food and different animal species according to data of Hungarian resistance monitoring system in 2001. *Acta Vet. Hung.* 51: 451-460.
- Lee H (2003). Methicillin (oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. *Appl. Environ. Microbiol.* 69: 648-6494.
- Quddoumi S, Bdour S, Mahasneh A (2006). Isolation and characterization of methicillin-resistant *Staphylococcus aureus* from livestock and poultry meat. *Ann. Microbiol.* 56: 155-161.
- Romero-Tabarez M, Jansen R, Sylla M, Luensdorf H, Huessler S, Santosa D, Timmis K, Molinari G (2006). 7-*O*-Malonyl macrolactin A, a new macrolactin antibiotic from *Bacillus subtilis* active against methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci and a small-colony variant of *Burkholderia cepacia*. *Antimicrob. Agents Chemother.* 50: 1701-1709.
- Saimann L, Tabibi S, Starner T, San Gabriel P, Winokur P, Jia H, McCray P, Tack B (2001). Cathelicidin peptides inhibit multiply antibiotic-resistant pathogens from patients with cystic fibrosis. *Antimicrob. Agent. Chemother.* 45: 2838-2844.
- Seguin C, Walker R, Caron J, Kloos W, George C, Hollis R, Jones R, Pfaller M (1999). Methicillin-resistant *Staphylococcus aureus* outbreak in a veterinary teaching hospital: potential human-to-human transmission. *J. Clin. Microbiol.* 37: 1459-1463.
- Sieradzki K, Roberts R, Haber S, Tomasz A (1999). The development Of vancomycin resistance in a patient with methicillin-resistant *Staphylococcus aureus* infection. *N. Engl. J. Med.* 340: 517-523.