Assiut Vet. Med. J. Vol. 58 No. 135 October 2012

ISOLATION OF *BURKHOLDERIA CEPACIA* COMPLEX FROM RAW MILK OF DIFFERENT SPECIES OF DAIRY ANIMALS IN ASSIUT GOVERNORATE

NAGAH M. SAAD and WALLAA F. AMIN
Department Food Hygiene, Faculty of Veterinary Medicine, Assiut University.

ABSTRACT

Received at: 29/9/2012

Accepted:

This study aimed to detect *Burkholderia cepacia* complex in raw milk samples of different dairy animals. A total of 120 raw milk samples of cow's, buffalo's, sheep's and goat's milk (30 samples each) were examined for the detection of *Burkholderia cepacia* complex (Bcc). It is evident from the approved results that a total of 31 raw milk samples (25.83%) were positive, representing 5 (16.66%) of buffalo's milk, 7 (23.33%) of cow's milk, 10 (33.33%) of sheep's milk and 9 (30%) of goat's milk. Therefore, contaminated milk may serve as a potential source of infection with *Burkholderia cepacia* complex which can cause life-threatening pulmonary infections in patients with chronic granulomatous disease or cystic fibrosis as they are opportunistic pathogens for humans. The resistance of randomly selected 10 Bcc isolated strains to five antibiotics was determined using the disc diffusion method, all isolates exhibited resistance to more than one antibiotic.

Key words: *Burkholderia cepacia complex, raw milk, opportunistic, antibiotic.*

INTRODUCTION

Burkholderia are gram negative non spore forming aerobic bacilli. They are versatile microorganisms that inhabit a wide variety of ecological niches as soil, water, animals and human respiratory tract (Coenye and Vandamme, 2003). *Burkholderia* is an important bacterial genus with a complex taxonomy that contains species of both ecological and pathogenic importance, including nine phenotypically similar species collectively termed *Burkholderia cepacia* complex (Bcc) (Luvizotto and Marcon, 2010). The genus *Burkholderia* was created by Yabuuchi (Yabuuchi *et al.*, 1992) and named after Burkholder who had discovered the genus but classified it as *Pseudomonas* (Burkholder, 1950).

Because the pseudomonads are commonly associated with the spoilage microflora of foods, various studies have previously identified *B. cepacia* complex as a spoilage organism in food. Moreover, Bcc have emerged as important life threatening opportunistic pathogen for human particularly in individuals with cystic fibrosis (LiPuma, 1998a) and patients with chronic granulomatous disease (Speert *et al.*, 1994). *B. cepacia* has also been identified as the causative agent in some cases of endocarditis (Hirose *et al.*, 1998) and nosocomial infection outbreaks (Kaitwatcharachai *et al.*, 2000).

Animal infections caused by Bcc have been reported (Berriatua *et al.*, 2001), but in general, its distribution in animal species and infection are not well documented.

There is a general consensus that the widespread use of antimicrobial agents has imposed a strong selective pressure that contributed to the emergence of multidrug resistant microorganisms (Levy, 2002). Since Bcc are resistant to most antimicrobial agents, effective therapies are not straightforward and management efforts are therefore aimed at prevention of infection (LiPuma, 1998b). Therefore, the aim of this study was to detect the incidence of *B. cepacia* in raw milk of different species of dairy animals, in order to assess its potentiality as a source of infection. Also, to study the resistance of Bcc to antibiotics, since they display high levels of resistance, therefore, infections with these microorganisms are difficult to treat and in some cases result in death.

MATERIALS and METHODS

A total of 120 raw milk samples of cow's, buffalo's, sheep's and goat's milk (30 samples each) were collected from different localities in Assiut Governorate, Egypt. The samples were transported to the laboratory at 4°C with a minimum of delay to be microbiologically examined.

Enrichment procedures: (Moore *et al.*, 2001)
Ten milliliters of samples were aseptically inoculated into 225 ml of nutrient broth and incubated at 30 °C for 24 hours.

Selective plating and identification of isolates:
Incubated broth cultures were streaked onto plates of *B. cepacia* selective agar (BCSA) as described by Henry *et al.* (1997). Plates were incubated at 30 °C for 48 hours followed by a further incubation at ambient temperature for 5 days. The isolates were identified by oxidase test, nitrate reduction test and glucose, lactose, sucrose fermentation (Cowan and Steel, 1974; Harrigan and McCance, 1976; A.P.H.A., 1992; Henry *et al.*, 2001).

Antimicrobial sensitivity test:
The antimicrobial sensitivity test and its interpretation were done using the disc diffusion method following the NCCLS standards (1997) for 10 Bcc isolates selected randomly. The following antimicrobial agents discs (Oxoid) were used to determine the pattern of resistance; ampicillin 10 µg, amoxycillin 10 µg, streptomycin 10 µg, gentamicin 10 µg and chloramphenical 30 µg.

RESULTS

Table 1: Incidence of *Burkholderia cepacia* complex in raw milk samples of different dairy animals species

Examined milk samples	No. of examined samples	Positive samples	
		No.	%
Buffalo's milk	30	5	16.66
Cow's milk	30	7	23.33
Sheep's milk	30	10	33.33
Goat's milk	30	9	30
Total	120	31	25.83

Table 2: Antibiotic sensitivity of *B. cepacia* complex isolates

Antimicrobials	Conc (µg/disc)	No. (%)	
		Resistant	Sensitive
Ampicillin	10	10(100%)	0
Amoxycillin	10	10(100%)	0
Streptomycin	10	3(30%)	7(70%)
Gentamicin	10	2(20%)	8(80%)
Chloramphenical	30	8(80%)	2(20%)

DISCUSSION

Presence of *Burkholderia cepacia* complex is not well documented in milk, so this study was carried out to investigate its presence in milk. In addition, this study aimed to detect Bcc in milk of different dairy animal species to have an overall prospective on its prevalence.

The detection of *B. cepacia* in raw milk (Uraz and Citak, 1998) and cheese (Smith *et al.*, 1987) were previously reported, indicating the possibility of food-borne spread to susceptible humans. The used selective agar medium, *B. cepacia* selective agar (BCSA) has proved to be the most effective selective agar for *B. cepacia* complex as it actually suppresses the growth of non *B. cepacia* bacteria (Henry *et al.*, 1999).

The recorded results in Table 1, show that Bcc was detected in 31 out of 120 raw milk samples (25.83%); representing 5 (16.66%) of buffalo's milk, 7 (23.33%) of cow's milk, 10 (33.33%) of sheep's milk and 9 (30%) of goat's milk. Higher incidence was reported by Moore *et al.* (2001), who reported that 14 of 26 (53.8%) samples of raw bovine milk were positive for *Burkholderia cepacia* complex. Its presence in raw milk could be attributed to the use of contaminated water or from animals suffering from mastitis. Berriatua *et al.* (2001) isolated Bcc from ewe's milk suffering from subclinical mastitis.

Moreover, it could contaminate the milk from the environment, and this could explain the higher incidence in sheep's and goat's milk than that of buffalo's and cow's. Since the samples of sheep's and goat's milk were collected from farmers' houses who most likely don't follow hygienic measures during milking and handling of milk. The detection of Bcc in raw milk samples is of great concern as it can survive in milk for a long time (Mohan Nair *et al.*, 2002).

Studying the antibiotic sensitivity of Bcc in Table 2, revealed that all isolates exhibited resistance to more than one antibiotic. All isolates were resistant to ampicillin and amoxicillin (100%), while 70%, 80%, 20% of the tested isolates were susceptible to streptomycin, gentamicin and chloramphenical, respectively. Isles *et al.* (1984) studied the antimicrobial sensitivity of Bcc isolates and found that they were resistant to ampicillin (97%), gentamicin (97%) and chloramphenicol (45%). Further surveys on Bcc species also observed this type of broad resistance (Nzula *et al.*, 2002 and Zhou *et al.*, 2007). Moreover, Mahenthiralingam *et al.* (2005) reported that Bcc is characterized by innate resistance to antibiotics.

CONCLUSION

Results of the present study indicated that *Burkholderia cepacia* complex were detected in raw milk samples of different dairy animals species. Therefore, raw milk is a potential source of infection. The presence of Bcc in milk is of great concern because of their capability to grow at low temperature and because Bcc have emerged as an opportunistic pathogen especially for patients suffering from cystic fibrosis that may lead to life-threatening infections. Bcc exhibit resistance to many antibiotics, and infected patients don't seem to respond to treatment. The eradication of Bcc as a human pathogen will become increasingly important.

REFERENCES

A.P.H.A. (American Public Health Association) (1992): Standard Methods for the Examination of Dairy Products. 16 th Ed., American Public Health Association.

Berriatua, E.; Ziluaga, I.; Miguel-Virto, C.; Uribarren, P.; Juste, R.; Laevens, S.; Vandamme, P. and Govan, J.R.W. (2001): Outbreak of subclinical mastitis in a flock of dairy sheep associated with *Burkholderia cepacia* complex infection. J. Clin. Microbiol, 39 (3): 990–994.

Burkholder, W. (1950): Sour skin, a bacterial rot of onion bulbs. Phytopath., 40: 115-117.

Coenye, T. and Vandamme, P. (2003): Diversity and significance of *Burkholderia* species occupying diverse ecological niches. Enviro. Microbiol., 5 (9): 719-729.

Cowan, S.T. and Steel, K.J. (1974): Manual for the Identification of Medical Bacteria. 2 nd Ed., Cambridge Univ. Press, England.

Harrigan, W.E. and McCance, M.E. (1976): Laboratory Methods in Food and Dairy Microbiology. Academic Press, London.

Henry, D.A.; Campbell, M.E.; LiPuma, J.J. and Speert, D.P. (1997): Identification of *Burkholderia cepacia* isolates from patients with cystic fibrosis and use of a simple new selective medium. J. Clin. Microbiol., 35: 614-619.

Henry, D.A.; Campbell, M.E.; Mcgimpsey, C.; Clarke, A.; Louden, L.; Burns, J.; Roe, M.; Vandamme, P. and Speert, D.P. (1999): Comparison of isolation media for recovery of *Burkholderia cepacia* complex from respiratory secretions of patients with cystic fibrosis. J. Clin. Microbiol., 37: 1004-1007.

Henry, D.A.; Mahenthiralingam, E.; Vandamme, P.; Coenye, T. and Speert, D.P. (2001): Biochemical and molecular approaches for determining genomovar status of the *Burkholderia cepacia* complex. J. Clin. Microbiol., 39: 1073- 1078.

Hirose, S.; Nakano, K.; Kosakai, Y.; Sasaki, T.; Kobayashi, J.; Sasako, Y.; Yamamoto, E.; Ueda, H.; Yutani, C. and Kitamura, S. (1998): Surgical treatment for prosthetic valve endocarditis. J. Cardiol., 31:85- 89.

Isles, A.; Maclusky, I.; Corey, M.; Gold, R.; Prober, C.; Fleming, P. and Levison, H. (1984): *Pseudomonas cepacia* infection in cystic fibrosis: an emerging problem. J. Pediatr., 104 (2): 206–210.

Kaitwatcharachai, C.; Silpapojakul, K.; Jitsurong, S. and Kalnauwakul, S. (2000): An outbreak of *Burkholderia cepacia* bacteremia in hemodialysis patients: An epidemiologic and molecular study. Am. J. Kidney Dis., 36: 199-204.

Levy, S.B. (2002): Factors impacting on the problem of antibiotic resistance. J. Antimicrob. Chemother., 49: 25-30.

LiPuma, J.J. (1998a): *Burkholderia cepacia* epidemiology and pathogenesis: implications for infection control. Curr. Opin. Pulm. Med., 4: 337- 441.

LiPuma, J.J. (1998b): *Burkholderia cepacia*: management issues and new insights. Clin. Chest Med., 19: 473- 486.

Luvizotto, D. and Marcon, J. (2010): Genetic diversity and plant-growth related features of *Burkholderia* spp. from sugarcane roots. World J. Microbiol. Biotechnol., 26: 1829-1836.

Mahenthiralingam, E.; Urban, T.A. and Goldberg J.B. (2005): The multifarious, multireplicon *Burkholderia cepacia* complex. Nat. Rev. Microbiol., 3: 144- 156.

Mohan Nair, M.K.; Vasudevan, P.; Hoagland, T. and Venkitanarayanan, K. (2002): Survivability of *Burkholderia cepacia* in pasteurized and unpasteurized bovine milk stored at 4°C and 8°C. Annual Meeting and Food Expo, Food Microbiology, General I, 61 C, Anaheim California, USA.

Moore, J.E.; McIlhatton, B.; Shaw, A.; Murphy, P.G. and Elborn, J.S. (2001): Occurrence of *Burkholderia cepacia* in foods and waters:

Clinical implications for patients with cystic fibrosis. J. Food Prot., 64 (7): 1076- 1078.

NCCLS (National Committee for Clinical Laboratory Standards) (1997): Approved standard M2-A6. Performance standards for antimicrobial disc susceptibility tests. 6th Ed. NCCLS, Wayne, PA.

Nzula, S.; Vandamme, P. and Govan, J. (2002): Influence of taxonomic status on the in vitro antimicrobial susceptibility of the *Burkholderia cepacia* complex. J. Antimicrob. Chemother., 50 (2): 265–269.

Smith, D.; Mikolajcik, E. and Lindamood, J. (1987): Causative organisms and chemical nature of the Swiss cheese rind rot defect. Cult. Dairy Prod. J., 22: 9–12.

Speert, D.P.; Bond, M.; Woodman, R.C. and Curnette, J.T. (1994): Infection with *Pseudomonas cepacia* in chronic granulomatous disease: role of nonoxidative killing by neutrophils in host defence. J. Infec. Dis., 170: 1523- 1531.

Uraz, G. and Citak, S. (1998): An investigation on the distribution and isolation of *Pseudomonas* from raw milk samples obtained from different areas. Turk. J. Agric. For. 22: 469–474.

Yabuuchi, E.; Kosako, Y.; Oyaizu, H.; Yano, I.; Hotta, H.; Hashimoto, Y.; Ezaki, T. and Arakawa, M. (1992): Proposal of *Burkholderia* gen. nov. and transfer of seven species of the genus *Pseudomonas* homology group II to the new genus, with the type species *Burkholderia cepacia* comb. nov. Microbiol Immunol., 36: 1251-1275.

Zhou, J.; Chen, Y.; Tabibi, S.; Alba, L.; Garber, E. and Saiman, L. (2007): Antimicrobial susceptibility and synergy studies of *Burkholderia cepacia* complex isolated from patients with cystic fibrosis. Antimicrob. Agents Chemother., 51 (3): 1085–1088.

عزل مجموعة البيركولديريا سيباشيا من اللبن الخام من الحيوانات الحلوبة المختلفة فى محافظة أسيوط

نجاح محمد سعد ، ولاء فاروق أمين

كان هدف هذه الدراسة هو عزل مجموعة البيركولديريا سيباشيا من اللبن الخام. تم فحص ١٢٠ عينة من اللبن الخام للحيوانات الحلوبة المختلفة فى محافظة أسيوط بواقع ٣٠ عينة لكل من اللبن الجاموسى والبقرى والأغنام والماعز. وقد تم فحص العينات لمعرفة مدى تواجد مجموعة البيركولديريا سيباشيا وقد أظهرت النتائج تواجد هذه المجموعة فى ٣١ عينة من اللبن الخام (25.83%) بواقع (16.66%) 5 للبن الجاموسى ، (23.33%) 7 للبن البقرى ، (33.33%) 10 للبن الأغنام و (30%) 9 للبن الماعز. ومن هذه النتيجة نستنتج ان اللبن الخام قد يكون مصدرا للعدوي بمجموعة البيركولديريا سيباشيا وتسبب عدوى رئوية لمرضى التليف الكيسى بما انها بكتيريا ممرضة انتهازية. وقد تم دراسة مقاومة ١٠ من العترات المعزولة لخمسة من المضادات الحيوية، وقد أظهرت الدراسة مقاومة كل العترات لأكثر من نوع من هذه المضادات الحيوية.