Isolation and Identification *Enterobacter asburiae* from Consumed Powdered Infant Formula Milk (PIF) in the Neonatal Intensive Care Unit (NICU)

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Received: 18 Dec. 2013; Accepted: 6 Jun. 2014

Abstract- *Enterobacter asburiae (E. asburiae)* is a facultative anaerobic, non-spore-forming gram-negative rod-shaped bacterium belonging to the family of *Enterobacteriaceae*. It is an opportunistic pathogen that its strains are isolated from a variety of clinical and environmental specimens. Since powdered infant formula milk (PIF) is not a sterile product, it is an excellent medium for bacterial growth. The aim of this study was to isolate and identify *E. asburiae* from PIF in the neonatal intensive care unit (NICU) and determine antimicrobial susceptibility patterns of this bacterium. A total 125 PIF samples were purchased from drug stores between June 2011 to March 2012. *E. asburiae* was isolated according to FDA method. For final confirmation, biochemical tests embedded in the API-20E system were used. The drug susceptibility test was performed using the disc diffusion method according to CLSI recommendations. Out of the 125 PIF samples investigated, 2 (1.6%) samples were positive for *E. asburiae*. All isolated strains were uniformly susceptible to aztreonam, cefotaxim, amikacin, streptomycin, nalidixic acid, meropenem, tetracycline, ceftazidime, and colistin. Variable susceptibility was seen to the some antimicrobial agents tested. Each country should categorize its own designed guidelines for the preparation and handling of PIF adapted to the local environment. Moreover, the pathogenesis of the *E. asburiae* in infants hospitalized in NICU and other groups such as immunosuppressed patients and HIV infected individuals is uncertain and requires further study.

Acta Med Iran 2016;54(1):39-43.

Keywords: Powdered infant formula milk (PIF); Neonatal intensive care unit (NICU); *E. asburiae*

Introduction

Enterobacter asburiae (E. asburiae) is a motile (by peritrichous flagella), facultative anaerobic, non-spore-forming gram-negative rod-shaped bacterium belonging to the family of *Enterobacteriaceae* (1-3). It is a new species in *Enterobacter* genus that was formerly called as Enteric Group 17. This organism was described and named by Brenner and coworkers in 1986 (4). *E. asburiae* is named in honor of "Mary Alyce Fife-Asbury" an American bacteriologist who made many important contributions to the taxonomy of the *Enterobacteriaceae* family (5). The DNA G + C content of *E. asburiae* is 55 ± 1.1 mol% (4).

E. asburiae have been isolated from soil and water and a variety of human sources including urine, respiratory tracts, stools, wounds, blood, endometrium, gall bladder, lochia exudate, penis, peritoneal fluid, and synovial fluid (4,6,7). This organism has been isolated from a wide variety of crops such as cotton, cucumber, common bean, and rice (2,8-10).

Some of the *E. asburiae* isolates are identified as human pathogens. *E asburiae* is an opportunistic pathogen and causes different human diseases such as community-acquired pneumonia, soft tissue infections, wound infection and other infections (3,4,11,12). *E. asburiae* produces the Bush group 1 β-lactamase enzyme constitutively at high levels which causes this bacterium be resistant to most β-lactam antibiotics (13).

This microorganism can cause opportunistic infections in immunocompromised (usually hospitalized) hosts. Before a baby formula was routinely screened for *Enterobacter*, many infants contracted bacterial infections due to this *Enterobacter's* ability to

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survive the heating preparations of the powdered formula.

Decreased transplacental transfer of maternal immunoglobulin G antibodies and reduced endogenous synthesis of IgG antibody predispose infants born prematurely to nosocomial infections. Neonates, particularly those who are preterm and low birth weight, are at higher risk of acquiring infections compared with the term and older infants. Patients in the neonatal intensive care unit (NICU) are one neonate group most at risk of acquiring healthcare-associated infections (15). Nosocomial infections cause significant morbidity and mortality in NICU hospitalized patients. Cross-transmission of micro-organisms, either directly from the hands or indirectly from environmental sources is a major contributing factor in the current infection threats to hospital inpatients (16).

Enterobacter spp. are the less common cause of hospital-acquired infections in NICUs. The members of this genus are multidrug resistant pathogens and can cause different diseases with high mortality rates in preterm infants. Several outbreaks caused by these opportunistic pathogens have been linked to intrinsically contaminate powdered infant formula (PIF) (17). Underlying diseases, low-birth-weight, immunocompromised immune system, cancer chemotherapy, and intravenous catheterization can be predisposing factors in cases of infections due to unusual microorganisms, including *E. asburiae* in neonates.

There are little information and evidence about the role of powdered infant milk formula in the transmission of opportunistic microorganisms to neonates and NICU hospitalized patients. Contamination of PIF with *E. asburiae* will be associated with the development of disease among neonates. Therefore, the microbiological safety of PIF is of most importance. Because PIF is not a sterile product, it is an excellent medium to support bacterial growth. Bovine milk and plant materials are essential ingredients of PIF and a potential source of various bacteria that are pathogenic to neonates and adults. The aim of this study was to isolate and identify *E. asburiae* from PIF in NICU in Tehran (Iran) hospitals and determine antimicrobial susceptibility patterns of this bacterium.

Materials and Methods

Sampling

A cross-sectional study was carried out on 125 PIF samples purchased from hospital drug stores between Jun 2011 to March 2012.

Isolation and Identification

In this study for isolation *Enterobacter asburiae* from PIF samples, the PIF cans were surface sterilized with 70% ethanol (Merck Co.) and were opened in a laminar flow cabinet. Samples were taken from each product under aseptically conditions. E. asburiae was isolated according to FDA method (18,19). We prepared 3 Erlenmeyer flask each of sterile distilled water (pre-warmed to 45ºC) at 9, 90 and 900 ml containing 1, 10 and 100 g of PIF, respectively. After the PIF was completely mixed and dissolved in distilled water, it was incubated at 37 ºC for 18-24 h. Following incubation, 10 ml of each sample was added to 90 ml of *Enterobacteriaceae* enrichment (EE) broth medium and placed at 37 ºC for 18-24 h. After incubation, a lapful of the enrichment culture was streaked onto duplicates violet red bile glucose agar (VRBGA) plates and cultured at 37 ºC for 18-24 h. A total of four suspicious colonies were picked from each VRBGA plate, and pure culture was performed. For detection non-lactose fermenting isolates, presumptive colonies were streaked onto MacConkey agar and incubated at 37ºC for 72 h. In order that final confirmation; biochemical tests embedded in the API-20E biochemical kit system (Bio-Mérieux) and manual biochemical tests were used according to the directions of the manufacturer. For long term storage, the purified isolates were saved in tryptic soy broth (TSB) with 20% glycerol (Merck Co.) at -20ºC.

Antibiotic sensitivity testing

Antibiotic sensitivity testing was done using Kirby-Bauer disk diffusion method on Mueller Hinton agar according to CLSI guidelines (20). Antimicrobial agents used in this study, were tetracycline (30µg), minocycline (30µg), tigecycline (15µg), imipenem (10µg), meropenem (10µg), aztreonam (30µg), ampicillin (10µg), mezlocillin (75µg), ticarcillin (75µg), carbenicillin (100µg), piperacillin (100µg), piperacillin-tazobactam (110µg), cefotaxime (30µg), ceftazidime (30µg), ceftriaxone (30µg), cefepime (30µg), ciprofloxacin (5µg), moxifloxacin (5µg), levofloxacin (5µg), streptomycin (10µg), gentamicin (10µg), amikacin (30µg), tobramycin (10µg), nalidixic acid (30µg), trimethoprim-sulfamethoxazole (25µg), colistin (25µg), and chloramphenicol (30µg).

Results

Out of the 125 PIF samples investigated, 2 (1.6%) of samples were positive for *Enterobacter asburiae*. The

gram staining of the colony of organism showed gram negative rods. On VRBGA agar selective medium purple/pink colored colonies, and on MacConkey agar lactose fermenting, smooth, convex, punctuate, umbilicated, glistening colonies were grown during 16 to 20 hours. Isolated strains were oxidase negative, catalase positive, motile and produced other biochemical reactions which are characteristic of *E. asburiae* (Table 1).

Table 1. Biochemical reactions of *Enterobacter asburiae*

Test	Reaction/Result
Gram stain	Gram-negative, rod
Triple Sugar Iron Agar	Acid (Yellow) slant/ Acid (Yellow) butt. No H2S
Motility	+
Oxidase	-
Catalase	+
Nitrate reduction	+
Simmons Citrate's at 37°C	+
Gas from Glucose	+
Acid from Glucose	+
Lactose	+
Maltose	+
Sucrose	+
Sorbitol	+
Mannitol	+
Xylose	+
Raffinose	+
Arabinose	-
D-Cellobiose	+
Malonate	-
Adonitol	-
Rhamnose	+
Inositol	+
Salicin	+
ONPG	+
Indole	-
Methyl Red (MR)	-
Voges Proskauer (VP)	+
Urease	-
Lysine decarboxylase	-
Ornithine decarboxylase	+
Arginine dehydrolase	+
DNAase	-
Esculin hydrolysis	-
Gelatin hydrolysis	-

All *E. asburiae* strains isolated from PIF samples were uniformly susceptible to aztreonam, cafotaxim, amikacin, streptomycin, nalidixic acid, meropenem, tetracycline, ceftazidime, ciprofloxacin, cefepime, imipenem, levofloxacin, piperacillin-tazobactam, cotrimoxazole, moxifloxacin, and colistin. Variable susceptibility was seen to the other antimicrobial agents tested (Table 2).

Table 2. Antimicrobial susceptibility pattern of *Enterobacter asburiae* strains isolated from PIF. (N=2)

Antibiotic	Sensitive (%)	Intermediate (%)	Resistant (%)
Ampicillin (AP)	1 (50)	-	1 (50)
Amoxicillin (A)	1 (50)	-	1 (50)
Aztreonam (ATM)	2 (100)	-	-
Cefotaxime (CTX)	2 (100)	-	-
Amikacin (AK)	2 (100)	-	-
Streptomycin (S)	2 (100)	-	-
Meropenem (MEM)	2 (100)	-	-
Mezlocillin (MEZ)	1 (50)	1 (50)	-
Nalidixic acid (NA)	2 (100)	-	-
Tigecycline (TGC)	1 (50)	1 (50)	-
Tetracycline (T)	2 (100)	-	-
Ticarcillin (TC)	1 (50)	-	1 (50)
Chloramphenicol (C)	-	1 (50)	1 (50)
Ceftazidime (CAZ)	2 (100)	-	-
Ciprofloxacin (CIP)	2 (100)	-	-
Cefepime (CPM)	2 (100)	-	-
Imipenem (IMI)	2 (100)	-	-
Levofloxacin (LEV)	2 (100)	-	-
Minocycline (MN)	1 (50)	-	1 (50)
Piperacillin (PRL)	1 (50)	-	1 (50)
Piperacillin-tazobactam (PTZ)	2 (100)	-	-
Carbenicillin (PY)	-	1 (50)	1 (50)
Tobramycin (TN)	1 (50)	-	1 (50)
Cotrimoxazole (TS)	2 (100)	-	-
Moxifloxacin (MFX)	2 (100)	-	-
Gentamicin (GM)	1 (50)	1 (50)	-
Colistin (CO)	2 (100)	-	-

Discussion

Many of the bacterial genera included known opportunistic pathogens that have been linked to outbreaks in NICUs. *E. asburiae* is an opportunistic pathogen that its strains arisolated from a variety of clinical and environmental specimens (4,6). Hospitalized infants in NICUs are particularly susceptible to opportunistic infections caused by bacteria, and infected neonates have high morbidity and mortality rates (21).

One of the biggest difficulties in preventing NICU ward and hospital-acquired infections is understanding the sources of the infectious agents and the routes of transmission. The consumption and use of PIF as infant food are wide-spreading; however, few studies have been conducted to evaluate *E. asburiae* role in food safety. Recent food-borne outbreaks traced to infant formula have raised concern about their processing and

handling. PIF, one of the most commonly used infant foods, has been identified as a source of food-borne illness.

To the best of our knowledge, there is no previous report on the isolation and identification *E. asburiae* from powdered infant milk formula milk and determination antimicrobial susceptibility patterns of this bacterium in Iran. Current study indicates that powdered infant milk formula samples are contaminated with *E. asburiae*. It has been isolated from different sources such as clinical specimens, foods, plants, in developed and developing countries (2,4,8-10). The current study showed all *E. asburiae* strains isolated PIF samples are uniformly susceptible to aztreonam, cafotaxim, amikacin, streptomycin, nalidixic acid, meropenem, tetracycline, ceftazidime, ciprofloxacin, cefepime, imipenem, levofloxacin, piperacillin-tazobactam, cotrimoxazole, moxifloxacin, and colistin. In present study, some isolates have variable susceptibility to the other antibiotics tested.

PIF are not sterile products and may contain microorganisms that can cause serious illness in infants, such as *E. asburiae.* Young children, especially infants, are vulnerable to foodborne illness. Babies can have difficulties fighting off infections because their immune systems are not yet fully developed. The infants who are at a higher risk of developing an *E. asburiae* infection include neonates, and those less than two months of age, especially, pre-term, low-birth-weight, HIV positive and immunocompromised infants with weakened immune systems. Infections caused by *E. asburiae* in PIF can be very serious and sometimes fatal.

PIF and other milk products are excellent media for regeneration and replication of potentially pathogenic and opportunistic bacteria. Producers report that, using current methods, it is not possible to omit all microorganisms from PIF in the manufactory (18-20). Inappropriate conditions of production, preparation, and handling of PIF may pose a substantial risk to neonates, preterm infants, and neonatal with weakened immune systems. Each country should categorize its own designed guidelines for the preparation and handling of PIF adapted to the local environment. Staff should be quite trained according to the planed guidelines, and educated in hygiene prescriptions for PIF preparation. Continuous monitoring accomplishment of the guidelines is necessary (22). Infections from resistant bacteria are now too common in worldwide (especially in developing countries), and some pathogens have even become resistant to different classes of antibiotics. Antibiotic-resistant infections can also come from the food (including PIF) we eat. The bacteria that contaminate food can be resistant because of the use of antibacterial in people and in food animals. HIV positive patients me (AIDS) are also at greater risk of invasive bacterial infections (e.g., foodborne pathogens) than the general population (23-26).

Public health institutes should affirm to parents, health care personnel and carries that PIF is not a sterile product, and good hygiene workouts are required in preparing PIF. Accurate consultation about the safe preparation, handling, and storage of PIF for health care workers in hospitals, especially the staff of intensive care units, and public health centers should be published separately. Moreover, the pathogenesis of the *E. asburiae* in infants hospitalized in an NICU and other groups such as immunosuppressed patients and HIV infected individuals is uncertain and requires further study.

Acknowledgment

This study was supported by Tehran University of medical sciences, Tehran, Iran.

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