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Full Length Research Paper

First report on *Enterobacter sakazakii* from Sudanese patients

Humodi A. Saeed[1]* and Rania M. Musallam[2]

Department of Microbiology, College of Medical laboratory Science, Sudan University of Science and Technology, Arbab El Agaid Street, P. O. Box 407 Khartoum, Sudan.

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***Enterobacter sakazakii* (*E. sakazakii*) has been identified as emerging opportunistic pathogens that can cause enterocolitis, bacteraemia, meningitis, brain abscess, and urinary tract infection. They have been particularly associated with meningitis in neonates where infant milk formulae have been epidemiologically linked to the disease. This study was carried out during the period of November 2008 to March 2009; to determine the occurrence of *E. sakazakii* in clinical specimens and its resistance to traditionally used antimicrobial agents in the Sudan. A total of 389 (311 urine specimens, 11 wound specimens, and 67 stool specimens) were collected from outpatients of three leading hospitals in Khartoum State. The urine specimens and wound swabs were cultured on blood and MacConkey's agars for primary isolation of pathogen, while stool specimens were cultured on selenite F broth and incubated overnight then subcultured on xylose lysine deoxycholate agar. Identification of the *E. sakazakii* was done by colonial morphology, Grams stain and biochemical tests using API 20E. Modified Kirby-Bauer disc diffusion method was adopted to determine the resistance rate of *E. sakazakii* to fifteen antimicrobial agents. Minimum inhibitory concentration (MIC) of antimicrobial agents was determined by E-test. The result showed that out of 389 specimens examined, 6 (1.5%) *E. sakazakii* were recovered, 4 (1.03%) from urine, 1(0.3%) from wound and 1(0.3%) from stool. The results more over revealed that the antimicrobial resistance of *E. sakazakii* was as follows; ceftazidime, amoxicillin, amoxyclav (100% each), co-trimoxazole, ticarcyline (83.3% each), chloramphenicol, tetracycline, ceftriaxone, nitrofuratoin, cephotaxime, tobramycin (66.7% each), ciprofloxacin, amikacin and nalidixic acid (16.7% each). None of the isolates were found to be resistant to gentamicin. In addition to that, the result indicated that the MIC, MIC_{50} and MIC_{90} of different antimicrobial agents range from 0.001 to > 240, 0.1 to > 240 and 0.5 to > 240 µg/ml respectively. The results indicated for the first time the presence of *E. sakazakii* in the examined clinical specimens in Sudan. The occurrence was high and the antimicrobial resistance of the isolated *E. sakazakii* was also high.**

Key words: *Enterobacter sakazakii,* API 20E, E-Test, Khartoum, Sudan.

INTRODUCTION

Enterobacter sakazakii (*E. sakazakii*) is a Gram-negative rod belonging to the family Enterobacteriaceae (Fiore et al., 2008). The organisms have been identified as emerging opportunistic pathogens that can cause enterocolitis, bacteraemia, meningitis, brain abscess and urinary tract infection. They have been particularly

associated with meningitis in neonates (Ongrádi, 2002; MacLean et al., 2009). Neonatal pathologies also include wound exudates, appendicitis, and conjunctivitis (Conte and Passantino, 2008). The organism can be found in broad range of foods including powdered infant formula, cheese, meat, vegetables, grain, herbs, spices, tomato, and in water, in a variety of areas, including hospitals and houses (Fiore et al., 2008; Kim et al., 2008; Beuchat et al., 2009).

Although most documented cases involve infant reports, infections in adult have been also described (Bhat et al., 2009). Adults with *E. sakazakii* infection usually have serious underlying disease or malignancies. The wound infection with *E. sakazakii* wound infection in an adult patient has been reported. The organism was resistant to multiple antibiotics and required prolonged treatment with broad spectrum antibiotics (Hamilton et al., 2003). It is thus necessary to introduce various control measures to reduce the risk of contamination at various levels (Fiore et al., 2008).

Antimicrobial agents from natural sources (muscadine seed extracts, rich sources of phenolic compounds and organic acids) demonstrated a strong antimicrobial effect against *E. sakazakii* (Kim et al., 2009). Control can be done using accurate antimicrobial agents. The later have been recovered from natural sources like, muscadine seed extracts, rich sources of phenolic compounds and organic acids to demonstrate a strong antimicrobial effect against *E. sakazakii* (Kim et al., 2009).

Cited publications have demonstrated that *E. sakazakii* can be isolated in hospitals from clinical specimens taken from patients. Revising literature, we found that this organism has never been isolated in the Sudan. This study was designed to determine possible occurrence and antimicrobial resistance of *E. sakazakii* in clinical specimens.

MATERIALS AND METHODS

Study design

This work is a descriptive and cross-sectional study that included 389 patients, complaining from symptoms of UTI, enterocolitis, and wound infection in Khartoum state. Urine, stool, and wound swab collection and patient's data were done in Kartoum Teaching Hospital (KTH), Gaffer Iben Auff Specialized Hospital for Children (GIASH) and Omdurman Teaching Hospital. Laboratory investigation was done in Research Laboratory, College of Medical Laboratories Science, Sudan University of Science and Technology. Data were collected in accordance with structured interview questionnaire.

*Corresponding author. E-mail: biotechsust@hotmail.com. Tel: +249 83773074. Fax: +249 83 771512.

Collection of specimens

Urine

Mid-stream urine (MSU) was collected. Patients were asked to clean the pre-urethra area with soap and water. Adult females were asked to make swabbing of the urethra with sterile swab avoiding any antiseptic then were asked to pass the first drops of urine and collect 10-20 ml of mid stream urine in sterile, wide mouth containers.

Wound swabs

Sterile cotton wool swab was used to collect specimens of discharge from the infected wound. Where there was no discharge, swab was moistened with sterile normal saline, prior to collection of specimen and subsequently inserted in sterile tube. In case of deeply ulcerated wound, a syringe was employed to aspirate the sample from the side wall of the ulcer. Specimens were delivered to the laboratory as soon as possible.

Stool

Freshly passed stool specimens were collected in sterile wide mouth containers and cultures were performed immediately after collection. In case of delay, specimens were stored at 4°C.

Cultivation of specimens

Under aseptic conditions, urine and wound specimens were streaked on MacConkey's agar and blood agar (Oxoid Ltd., UK). Stool specimens were cultured in selenite F broth (Oxoid Ltd., UK), incubated overnight at 37°C then subcultured on xylose lysine deoxycholate agar (XLD), (Oxoid Ltd., UK) and incubated aerobically overnight at 37°C.

Examination of growth

Abundant growth was examined for lactose fermentation on MacConkey's agar, XLD agar and haemolysis on blood agar. The various morphological characters of the targeted organism were also observed and recorded. The isolates were then streaked on nutrient agar and incubated overnight at 37°C. The growth was checked for purity and stored in Bijou Bottles for further investigations.

Criteria of isolation of *E. sakazakii*

Gram-negative rods were considered significant (Guptta et al., 2003) and were investigated.

Identification of *E. sakazakii*

Bacterial colonies were examined for round, irregular, crenated or branching appearance, transparency or opacity, smoothness or roughness, dullness or shiny appearance. The color of the colonies was also examined (Murray et al., 2007).

Gram's stain

The method was carried out according to Murray et al. (2007).

Oxidase test

The test was carried out according to Barrow and Filtham (2003). Briefly, oxidase reagent discs were placed on sterile Petri dish and rubbed with colony of the test organism removed by using a wooden stick. Formation of a red-purple color within 20 s indicated positive oxidase test.

Analytical profile index (API 20E)

The API 20E (20 biochemical tests) was performed according to manufacturer instructions. These were ONPG, ADH, LDC, ODC, CIT, H_2S, URE, TDA, IND, VP, GEL, GLU, MAN, INO, SOR, RHA, SAC, MEL, AMY and ARA. Identification was obtained with the numerical profile and performed using the database with the analytical profile index by looking up to the numerical profile in the list of profile.

Antimicrobial sensitivity test

Modified Kirby-Bauer disc diffusion method was performed (WHO, 1996; NCCLS. 1997; Murray et al., 2007). Briefly, plates of Mueller-Hinton agar (Oxoid Ltd, UK) were prepared according to the manufacturer instructions; the sterilized molten medium was cooled to 45 to 50°C and poured in sterile, dry Petri plates on a leveled surface, to a depth of 4 mm. The inoculum was prepared and turbidity was adjusted to 0.5 McFarland standard. A sterile non-toxic cotton swab on a wooden applicator was dipped into the standardized inoculum and the soaked swab was rotated firmly against the upper inside wall of the tube to express excess fluid. The entire agar surface of the plate was streaked with the swab three times with turning the plate at an angle of 60 degrees between each streaking; the inoculum was allowed to dry for 5-15 min with lid in place. Using sterile forceps the antimicrobial discs were placed and evenly distributed on the inoculated plate. The plate was then inverted and incubated aerobically at 35-37°C for 18-24 h. After overnight incubation, the control and test plates were examined to ensure the growth is confluent or near confluent. Using a ruler on the under side of the plate, the diameter of each inhibition zone was measured in (mm).

Interpretation of the zone size

Using the interpretive chart, the inhibition zone around each antibiotic disc was measured and reported as sensitive, resistant or intermediate according to WHO (1996), NCCLS (1997) and Murray et al. (2007).

Minimum inhibitory concentration (MIC) test

The MIC test was performed to each isolate by the E-test technique as recommended by the manufacturer (HiMedia Laboratories Pvt. Limited). Briefly, each isolate was tested against the fifteen antimicrobial agents. A 200 mm Petri plate containing 90 ml of Mueller-Hinton agar (M173) was used. The inoculum was prepared as suspension in 0.85% NaCl. The suspension was adjusted to a 0.5 McFarland standard. Plates were seeded with a cotton swab as described by manufacturer. The E-test strips were applied after the excess moisture had been absorbed into the agar. The plates were then incubated at 35-37°C and examined after 18-24 h.

RESULTS

During the course of this study (November 2008 to March 2009) a total of 389 patients were recruited. 311 of these, presented with UTI, 11 with wound infection and 67 with enterocolitis. Out of the total number of urine samples collected, 163 (52.4%) showed significant bacterial growth (127 lactose fermeters (LF) and 36 non-lactose fermeters (NLF)). 10 (90.9%) of wound swabs and 61 (91%) of stool specimens revealed significant growth, 4 LF; 6 NLF and 61 LF 6 NLF respectively.

Identification of *E. sakazakii*

Colonial morphology

Colonial morphology was observed on blood and MacConkey's agars after overnight incubation for urine and wound specimens, and on XLD for stool. Suggestive *E. sakazakii* isolates produced large (3-4 mm), dull-gray, and dry or mucoid colonies on blood agar, large and pink or colorless, mucoid colonies on MacConkey's agar and

Table 1. Criteria for identification *E. sakazakii* on API 20 E.

Biochemical tests	Reaction	Result
ONPG	Yellow-pale yellow	Positive
ADH	Red / orange	Positive
LDC	Yellow color	Negative
ODC	Red / orange	Positive
CIT	Blue-green blue	Positive
H_2S	Colorless	Negative
URE	Yellow color	Negative
TDA	Yellow color	Negative
IND	Colorless	Negative
VP	Pink/ red	Positive
GEL	Diffusion of black pigment	Positive
GLU	Yellow color	Positive
MAN	Yellow color	Positive
INO	Blue/ blue-green	Negative
SOR	Blue/ blue-green	Negative
RHA	Yellow color	Positive
SAC	Yellow color	Positive
MEL	Yellow color	Positive
AMY	Blue/ blue-green	Negative
ARA	Yellow color	Positive

Table 2. Antimicrobial resistance of *E. sakazakii* isolates (n=6).

Antibiotic	Resistance (%)
Chloramphenicol	66.7
Ciprofloxacin	16.7
Tetracycline	66.7
Gentamicin	00.0
Ceftazidime	100.0
Amikacin	16.7
Ceftriaxone	66.7
Ticarcyline	83.3
Nitrofuratoin	66.7
Co-trimoxazole	83.3
Amoxicillin	100.0
Amoxyclav	100.0
Nalidixic acid	16.7
Cefotaxime	66.7
Tobramycin	66.7

pink color on XLD.

API 20 E

The results of API 20E revealed that ONPG, ADH, ODC and CIT were positive, H_2S, LDC, URE, TDA and IND were negative. VP and GEL were also positive and all sugars GLU, MAN, RHA, SAC MEL, AMY and ARA were positive except INO, SOR, AMY which were negative (Table 1).

The total number of *E. sakazakii* isolates were 6 (1.5%) including 4 (1.03%) from urine, 1 (0.3%) from wound and 1(0.3%) from stool. The results revealed that the resistance rate of *E. sakazakii* was (100%) to amoxicillin and amoxyclav, ceftazidime, (0%) to gentamicin, (83.3 %) to co-trimoxazole and ticarcyline, (16.7%) to ciprofloxacin, amikacin, and nalidixic acid, (66.7%) to chloramphenicol, tetracycline, nitrofuratoin, cefotaxime, ceftriaxone, and tobramycin (Table 2). The Minimum Inhibitory Concentration (MIC), MIC_{50} and MIC_{90} values respectively were (3->240, 60 and >240 µg/ml) for nitrofurantoin, (0.5->240, 4 and 4 µg/ml) for nalidixic acid, (0.1->240, >240 and >240 µg/ml) for co-trimoxazole, (0.01->240, 4 and 4 µg/ml) for chloramphenicol, (0.1-64, 0.1 and 0.5 µg/ml) for amikacin, (0.1-30, 5 and 30 µg/ml) for gentamicin, (1-16, 4 and 8 µg/ml) for tobramicin, (0.001-0.5, 0.25 and 0.5 µg/ml) for ciprofloxacin, (0.1-60, 30 and 30 µg/ml) to ceftriaxone, (0.1->240, 15 and 15 µg/ml) for ceftazidime, (0.01-3, 2 and 3 µg/ml) for tetracycline, (10->240, >240 and >240 µg/ml) for ticarcycline, (0.1->240, >240 and >240 µg/ml) for amoxicillin, (0.1->240, 60 and 60 µg/ml) for cefotaxime, (2->240, >240 and >240 µg/ml) for amoxyclav (Table 3).

DISCUSSION

This study was conducted to determine the frequency and antimicrobial resistance of *E. Sakazakii*. Three hundred and eleven urine specimens, eleven wound specimens, and sixty seven stool specimens were investigated. Of these, 163 (52.4 %) from urine specimens, 10 (90.9%) from wound specimens, and 61(91%) from stool specimens showed significant various bacterial growth.

E. sakazakii was isolated from 6(1.5%) out of 389

Table 3. MIC range, MIC_{50} and MIC90 of antimicrobial agents to *E. sakazakii.*

Antibiotics MIC µg/ml	No tested	MIC µg/ml		
		MIC range	MIC_{50}	MIC_{90}
Nitrofuratoin	6	3 to >240	60	>240
Nalidixic Acid	6	0.5 to> 240	4	4
Co-trimoxazole	6	0.1 to > 240	>240	>240
Chloramphenicol	6	0.01 to > 240	4	4
Amikacin	6	0.1 to 64	0.1	0.5
Gentamicin	6	0.1 to 30	5	30
Tobramicin	6	1 to >16	4	8
Ciprofloxacin	6	0.001 to >0.5	0.25	0. 5
Ceftriaxone	6	0.1 to > 60	30	30
Ceftazidime	6	0.1 to > 240	15	15
Tetracycline	6	0.01 to 3	2	3
Ticarcyline	6	10 to > 240	>240	>240
Amoxycillin	6	0.1 to > 240	>240	>240
Cefotazime	6	0.1 to > 240	60	60
Amoxyclav	6	2 to > 240	>240	>240

MIC; Minimum inhibitory concentration.

patients (urine, wound and stool). The isolates showed large, dull-gray, and dry or mucoid colonies on blood agar and large, pink, mucoid colonies on MacConkey's agar. These results were comparable to those described by Erickson and Kornacki (2002) and Engelkirk and Duben-Engelkirk (2007). The frequency 6 (1.5%) of *E. sakazakii* in three types of infections (UTI, Enterocolitis, Wound infection) is similar to the results obtained by Paterso et al. (2005), who reported that *E. sakazakii* was responsible for (0.4%) of the three types of infection in five international regions including Asia, Europe, Latin America, North America, and Middle East.

In vitro activities of antimicrobial agents indicated that the resistance rate of *E. sakazakii* to amoxicillin and ceftazidime were (100%). This result is consistent with Farajnia et al. (2009) who found that the resistance rate of *E. sakazakii* to amoxicillin was (100%) in Iran but different from those of Sader et al. (2005) who found the resistance rate to ceftazidime was (17.5%) in United States. The resistance rate of *E. sakazakii* to nitrofuratoin (66.7%), however is widely different from those of Farajnia et al. (2009) who reported a 37.5% resistance rate to nitrofuratoin in Iran.

The efficacy of cefotaxime, tobramycin, gentamicin and ciprofloxacin were evaluated and the result showed the resistance rates of *E. Sakazakii* to these antibiotics was (66.7%), (66.7%), (0%) and (16.7%) respectively. This differs from results obtained by Pfaller and Jones (2002), who reported that the resistance rate to cefotaxime, tobramycin and gentamicin is (23%), (16%) and (11%) respectively but similar to ciprofloxacin (16%) in European countries. However, the results indicated that resistance to nalidixic acid (16.7%) was different compared to that of Farajnia et al. (2009) who reported a resistance rate of 0.0 % to this antibiotic. Similarly the resistance rate to amikacin in this investigation is quite different from (0.0%) resistance rate reported by Farajnia et al. (2009).

On the other hand, this study showed that the MIC_{50} and MIC_{90} of amikacin, ciprofloxacin, tobramycin, and gentamicin were in agreement with Pfaller and Jones (2002).

It is concluded that resistance rate of *E. sakazakii* is high to commonly used antimicrobial agents. It is also concluded that gentamicin is the first choice for treatment of *E. sakazakii* infections. Ciprofloxacin, amikacin, and nalidixic acid are the second choice of antimicrobial therapy with resistance rate of (16.7%). Nitrofurantoin,

tetracycline, ceftriaxone, cephotaxime, chloramphenicol, and tobramycin on the other hand are less effective antimicrobial agents due to their high resistance rate (66.7 %). Amoxicillin, amoxyclav, and ceftazidime are not effective, having a resistance rate of 100 %. However; further future studies including larger sample sizes are recommended, and to our knowledge this report represents the first record of *E. sakazakii* in the Sudan.

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REFERENCES

Barrow GI, Feltham RKA (2003). Cowan and Steel's Manual for the Identification of Medical Bacteria: 3rd edition; Cambridge University Press, p. 352. ISBN: 0521543282.

Beuchat LR, Kim H, Gurtler JB, Lin L, Ryu J, Richards GM (2009). *Cronobacter sakazakii* in foods and factors affecting its survival, growth, and inactivation, Int. J. Food. Microbiol., 2: 570-749.

Bhat G, Anandhi RS, Dhanya VC, Shenot MS (2009). Urinary tract infection due to *Enterobacter sakazakii*. India J. Pathol. Microbiol. 52(3): 430-431. DOI: 10.4103/0377-4929.55017.

Conte F, Passantino A (2008). Isolation of *E. sakazakii* from ass' milk in Sicily: Case report, safety and legal issues, 2nd Int Conference. J. Travel Med. Infect. Dis., 6(4): 250-252.

Engelkirk PG, Duben-Engelkirk J (2007). Laboratory Diagnosis of Infectious Disease: Essentials of Diagnostic Microbiology, Edition illustrated, Lippincott Williams and Wilkins, Philadelphia, USA. Ch, 12: 307-308.

Erickson MC, Kornacki JL (2002). *Enterobacter sakazakii*: an emerging food pathogen, J. Center. Food Safety, pp. 2-8.

Farajnia S, Alikhani MY, Ghotaslou R, Naghili B, Nakhband A (2009). Causitive agents and antimicrobial susceptibilities of urinary tract infections in the northwest of Iran, Int. J. Infect. Dis., 13(2): 140-144.

Fiore A, Casale M, Aureli P (2008). *Enterobacter sakazakii:* epidemiology, clinical presentation, prevention and control. Ann Ist Super Sanità, 44(3): 275-280.

Guptta V, Yadav A, Joshi RM (2003). Antibiotics Resistance Pattern in uropathogen Indian. J. Med. Microbial., 20: 96-98.

Hamilton JV, Lehane J, Braig HR (2003). Isolation of *Enterobacter sakazakii* from midgut of stomoxys calcitrans, J. Emerg. Infect. Dis., 9(10): 1355-1356.

Kim JB, Cho Seund-Hak JC, Park YB, lee JB, lec Kim JC, Lee BK (2008). Surveillance of stool samples for the presence of *E. sakazakii* among Korean people, Yonsei Med. J., 49(6): 1017-1022.

Kim TJ, Silva JL, Weng WL, Chen WW, Corbitt M, Jung YS, Chen YS (2009). Inactivation of *E. sakazakii* by water-soluble muscadine seed extracts, Int. J. Food. Microbiol., 129(3): 295-299.

MacLean LL, Pagotto F, Farber JM, Perry MB (2009). The structure of the O-antigen in the endotoxin of the emerging food pathogen *Cronobacter (Enterobacter) muytjensii* strain 3270, J. Carbohydrate Res., 344(5): 667-671.

Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA (2007). Manual of Clinical Microbiology. 9th ed. Washington DC: ASM Press, 2007.

NCCLS (1997). Performance standards for Antimicrobial disk Susceptibility tests. Six edition. ISBN 1 56238 306 6. Available from NCCLS. 940 West Valley Road. Suit 1400. Wayne. PA 19087-1898, USA.

Ongrádi J (2002). Vaginal infection by *Enterobacter sakazakii*. Sex Transm Infect., 78: 467.

Paterso DL, Rsossi F, Baquero F, Hsueh P, Woods GL, Satishchandran V, Snyder TA, Harvey CM, Teppler H, Dinubile MJ, Chow JW (2005). *In vitro* susceptibilities of aerobic and facultative Gram-negative bacilli isolate from patients with intra-abdominal infection world wide with 2003 Study for Monitoring Antimicrobial Resistance Trends (SMART), J. Antimicrob. Chemother., 33(6): 965-973.

Pfaller MA, Jones RN (2002). MYSTIC Study Group (Europe) Antimicrobial susceptibility of inducible Ampc B-lactamase-producing Enterobacteriaceae from the Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) Programme Europe 1997-2000, Int . J. Antimicrob. Agents, 19(5): 383-388.

Sader HS, Fritsche TR, Jones RN (2005). Potency and spectrum trends for cefepime tested against 65746 clinical bacterial Isolates Collected in North American Medical Centers: Results from the SENTRY Antimicrobial Surveillance Program (1998-2003), J. Diagnostic Microbiol. Infect. Dis., 52(3): 265-273.

WHO (1996). Guideline for antimicrobial resistance Surveillance, ISBN 92 9021 2136. Available from WHO Regional Office for the Eastern Mediterranean. PO Box 151. Alexandria 21511. Egypt.