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Original article

Intestinal carriage of methicillin resistant *Staphylococcus aureus* and extended -spectrum β-Lactamase- producing *Enterobacteriacae* in hospitalized and nonhospitalized patients and their clinical implications

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Abstract

Objective: To determine the clinical implication of and intestinal carriage with methicillin resistant *Staphylococcus aureus* (MRSA) and extended spectrum β-lactamase (ESBL)-producing *Enterobacteriacae*. **Methods:** A total of 180 stool specimens were screened for MRSA and ESBL-producing enterobacteria. Identification of ESBL- producing *Enterobacteriacae* was done by MicroScan Walk Away 96 system (Dade Behring Inc., West Sacramento, CA 95691, USA) and confirmation by double-disc synergy test. MRSA was identified by disc diffusion using 30 μg cefoxitin disc and the MicroScan. **Results:** The rate of fecal MRSA carriage was 7.8% (14/180), 35.7% (5/14) were recovered from surgical wards. Three patients (21.4%) had MRSA recovered from other body sites, and 2 (14.2%) had in addition ESBL -producing *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) respectively. Four (28.5%) patients with MRSA fecal carriage died. MRSA fecal carriage was recovered from both inpatients and outpatients. Four (2.2%) cases carried ESBL-producing *Enterobacteriacae* in feces. Three (75%) were from intensive care unit (ICU). One patient had both ESBL-producing *E. coli* and *K. pneumoniae* from stool as well as *E. coli* from tracheal aspirate. Two ICU patients with fecal ESBL died. **Conclusion:** Fecal screening for MRSA and ESBL of all patients at high risk admitted to different hospital wards and ICUs and implementing infection control measures were recommended.

Keywords: Intestinal carriage; Methicillin resistant; Staphylococcus aureus; Extended spectrum β-lactamase; Enterobacteriacae

INTRODUCTION

The emergence of antimicrobial resistance among nosocomial bacteria has gained a global concern. MRSA and *Enterobacteriacae* producing new types of ESBLs have been the two commonly reported topics in literature during the last few years[1-5]. These have resulted in an important infection control implications and being a major cause of healthcare-associated infections. Although both are most frequently transmitted in hospital settings; however, in recent years there has been several reports of true community acquired infections or colonization with these organisms[4-7]. Screening of patients at high risk of colonization by ESBL and MRSA has been recommended for controlling transmission of these organisms in hospitals. Culture of specimens from different body sites such as axillae, groin, perineum, anterior nares, wounds as well as other body sites often performed to identify colonized patients. However, screening fecal specimens for ESBL or MRSA carri-

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age is not routinely performed in our institution. Gastrointestinal carriage of ESBL or MRSA have been reported in nosocomial outbreaks, in non-nosocomial situations, as well as within the community[8, 11]. Previous studies have documented that at least 80% of the patients infected with ESBL-producing *K. pneumoniae* had prior gastrointestinal tract carriage and infection by this organism was reported to develop within weeks of acquiring gastrointestinal colonization[3, 12,13]. In addition, colonized patients may act as a potential source of transmission of these organisms within the hospital particularly those without signs of overt infection[3]. We conducted a prospective surveillance study on fecal specimens submitted to the microbiology laboratory at King Khalid University Hospital (KKUH) from all patients (hospitalized patients and outpatients) with the aim to determine the intestinal carriage with MRSA and ESBL among isolates of *S. aureus* and *Enterobacteriacae*, respectively, and their clinical implications.

MATERIALS AND METHODS

This study was conducted at the clinical microbiology laboratory of KKUH, Riyadh, Saudi Arabia over a three month period starting from June 1st, 2007 through August 31th 2007. KKUH is 850- bed primary, secondary and tertiary care hospital with five different intensive care units. We screened all stool specimens submitted to the laboratory from hospitalized patients (hospitalized for >72 hours including patients known to have ESBL or MRSA from sites other than stool) and outpatients suffering from diarrhea or abdominal pain for the presence of ESBL-producing Enterobacteriacae and MRSA. Patients´ data (including age, gender, inpatient / outpatient,) were obtained from clinical microbiology database, which included in addition previous isolation of ESBL or MRSA from other specimens. Non-repetitive stool specimen was taken from each patient.

Microbiological methods

From each submitted stool specimen , a portion was taken with a sterile cotton swab and plated into MacConkey agar plate (for *Enterobacteriacae*) and mannitol salt agar plate (for *S . aureus*) and incubated aerobically overnight at 37 ℃ . Plates that show no growth were further incubated for 24 hours. Lactose fermenting (pink) colonies from MacConkey agar plates that were oxidase negative were selected for further identification and susceptibility testing by the MicroScan. If the specimen contained lactose fermenting colonies with different morphologies, each morphotype was identified separately and tested for susceptibility. Specimens that show possible ESBL from the MicroScan were confirmed by double- disc synergy test. Mueller Hinton agar (Mueller Hinton, Becton Dickinson, USA) plates were inoculated with suspected isolates. Ceftazidime (30μg), cefotaxime (30μg), ceftriaxone (30μg), azteronam (30μg) were placed 30mm (center to center) from amoxicillin-clavulanate (20 μg and10 μg) disc as recommended by Jarlier et al[14]. After incubation, ESBL was indicated by enhancement of the zone of inhibition of the ESBL antibiotics nearer to amoxicillin-calvulanic acid disc (included quality control strains were *K. pneumoniae* strain ATCC 700603 positive control and *E. coli* strain ATCC 25922 negative control). Detected ESBLs were not further characterized to a molecular level. Yellow colonies from mannitol salt agar plate were further identified by coagulase and DNAse tests and tested for susceptibility by the MicroScan. Oxacillin susceptibility was tested by disc diffusion using cefoxitin 30 μg disc (Oxoid Basingstoke, Hampshire, England) on Mueller Hinton agar plates supplemented with 2% NaCl (Mueller Hinton, Becton Dickinson, USA). Oxacillin resistance was determined according to clinical laboratory standard institute (CLSI) guideline[15].

RESULTS

During the study period a total of 180 non-repetitive stool specimens from hospitalized and non hospitalized patients were included in our analysis. Summary of cases of MRSA and ESBL fecal carriage are presented in Table 1 and Table 2 respectively. Among those, 7.8% (14/180) were gastrointestinal carriers of MRSA. Nine of them were inpatients. Of them, five (35.7%) were from surgical wards and 2 (14.2%) were from ICU. Three (21.4%) patients had MRSA recovered from other body sites while two (14.2%) had ESBL- producing *E. coli* and *K. pneumoniae* (respectively) recovered from stool as well, Table 1. Four (28.5%) patients with MRSA intestinal carriage died, two of them were from surgical wards. MRSA intestinal carriage was from both hospitalized and non-hospitalized patients 9 (64.2%) and 5(35.7%), respectively.

Table1 Summary of cases of MRSA gastrointestinall carriage.

Case no.	Age/sex	location	MRSA other site	Other stool isolates	outcome
1	71 Y, M	Inpatient(Surgical ward)		*E. coli* (ESBL)	Not known
2	73 Y, M	Inpatient (ICU)		*K. pneumoniae*(ESBL)	Died
3	38 Y, F	Outpatient		*E. coli* (non-ESBL)	Not known
4	70 Y, M	Inpatient (Surgical ward)			Died
5	63 Y, M	Inpatient (Surgical ward)	Blood & Sputum		Died
6	1Y, F	Outpatient		*Salmonella spp.* & *E. coli* (non-ESBL)	Not known
7	73 Y ,M	Inpatient (Surgical ward)		*E. coli* (non-ESBL)	Died
8	1M, M	Intpatient (ICU)	Nose	*E. coli* (non-ESBL)	On therapy
9	9Y, F	Outpatient		*E. coli* & *K. pneumoniae* (non-ESBL)	Not known
10	2Y, M	Inpatient (Pediatric ward)		*E. coli* (non-ESBL)	Not known
11	20Y, F	Outpatient		*E. coli* (non-ESBL)	Not known
12	55Y, M	Outpatient		*E. coli* (non-ESBL)	Not known
13	2Y, F	Inpatient(Pediatric ward)		*E. coli* (non-ESBL)-	Not known
14	34Y, M	Inpatient(surgical ward)	Wound	*E. coli* (non-ESBL)	On local therapy
Total N(%)	14 (7.8)				

Table 2 Summary of cases of ESBL gastrointestinal carriage.

Case no.	Age/sex & location	ESBL producing organism	ESBL other site	Other stool isolates	outcome
1	2Y, M, ICU	*E. coli* & *K. pneumoniae*	Tracheal aspirate(E. coli)	*Citrobacter* spp. (non-ESBL)	Died
2	73Y, M,ICU	*K. pneumoniae*		MRSA	Died
3	71Y, M,Surgical ward	*E. coli*		MRSA	Not known
4	4Y, F,ICU	*Enterobacter cloacae*			Not known
Total N(%)	4 (2.2)				

Only 4 (2.2%) patients had ESBL- producing organisms recovered from stool screening, Table 2. Among these, three (75 %) patients were from ICU and the remaining was from surgical ward. None of ESBL- positive patients were from out side the hospital. One patient had both *E. coli* and *K. pneumoniae*-producing ESBL from stool as well as *E. coli* -producing ESBL from tracheal aspirate while two patients had MRSA recovered from stool along with ESBL-producing isolates . Two (50%) patients died were from ICU , one of them was an elderly patient who had both MRSA and ESBL producing- *K. pneumoniae* from stool.

Among the negative stool specimens, 18 (10%) of the patient (2 were inpatients) had only *Enterobacter sakazaki* isolated from stool specimens and 5 (2.8%) had *Citrobacter* species (one was an inpatient and had both ESBL- producing *E. coli* and *K. pneumoniae*).

DISCUSSION

Screening for gastrointestinal carriage with MRSA or ESBL-producing *Enterobacteriacae* is not routinely carried out during an outbreak situation or admission of the patient to an ICU in many hospitals including ours. In our study, all cases with gastrointestinal carriage of ESBL -producing *Enterobacteriacae* were hospitalized patients, this is in contrast to Valverde, et al study where the prevalence of fecal carriage of ESBL -producing *Enterobacteriacea* among outpatients was 5.5% compared to hospitalized patient (11.8 %)[11]. In Spain, the incidence was (7.5%) among outpatients[16]. However, Kader, et al reported 71(26.1%) fecal carriage of ESBL-producing isolates among inpatients and 25 (15.4%) a-

mong outpatients[8]. The majority of hospitalized patients in our study were from ICU (75%). The reported higher prevalence of fecal carriage of ESBL - producing Gram negative bacteria in ICU is explained by increasing length of stay in ICU and antibiotic selection pressure in hospitals which may amplify the number of carriers harboring such isolates[12,17]. Several studies have found a relationship between the use of third generation cephalosporins and /or fluoroquinolones and acquisition of an ESBL-producing organisms[3, 12, 17,18]. The clinical implications of gastrointestinal colonization with ESBL-producing organisms is that it is a prerequisite for infection by ESBL-producing organisms[8].

Reports are emerging about the increasing ESBL gastrointestinal carriage among community population that increased the risk of human-to human transmission of resistant organisms or through the environment[8, 19]. This could be explained by previous nosocomial acquisition and prolonged carriage of ESBL - producing bacteria over prolonged period[8]. Other explanation is that predisposing factors for acquiring ESBL among community patients exist including ; age over 60 years, diabetes, or neurological diseases and bed-ridden conditions[7]. Other factor is that many human-source drug-resistant fecal *E. coli* isolates more likely originated in poultry than in human which has many concerns regarding the potential human health risk for antimicrobial drug in poultry production[20]. Fortunately, none of our isolates of ESBL came from fecal specimens of non-hospitalized patient. However, gastrointestinal reservoir of ESBL-producing bacteria in the hospital may grow in the future leading to dissemination of these organisms into the community.

The resistance pattern of *Enterobacter cloacae* in our study was compatible with a chromosomally encoded AmpC ESBL. For that, an emerging AmpC β-lactamase- hyperproducing *E. coli* in the community with public health implications was reported[21].

In a similar study to ours, it showed 16.3% and 13.7% gastrointestinal carriage rate of MRSA and third-generation cephalosporins resistance among hospitalized patients, respectively[22]. Among these patients, 38.2% were reported to have infectious complications during their hospital stay including bacteremia, spontaneous bacterial peritonitis or urinary tract infection[22]. In our study, elderly, pediatric, ICU and surgical patients who were fecally colonized with MRSA or ESBL or both or had *MRSA* in the blood or at other body sites were more at risk of death compared to the other patients. This may reflects the severity of their illness and their need of prolonged hospitalization and antibiotic use which further increased the risk of acquiring *MRSA*. The intestine seems to be the source of MRSA in patients who do not have MRSA at other sites . Report on MRSA gastrointestinal carriage have indicated that patients infected with MRSA were often colonized with the same organism in the nares and rectum and that rectal carriage is more common than nasal carriage[23]. Although, the anterior nares are considered the best single body site to detect the source for MRSA colonization, gastrointestinal carriage occurs in a substantial proportion of patients with MRSA nasal colonization[24]. In addition, more than half of the patients with gastrointestinal carriage had no history of MRSA colonization or infection and that 80% of patients whose stool cultures yielded the first evidence of MRSA colonization were not being cared for contact precautions[24]. Transmission of MRSA from such patients is estimated to occur nearly 15 times as frequently as from patients who cared for using barrier precautions[24]. The importance of gastrointestinal carriage with MRSA in predicting to post-operative infection has not been stressed previously and so MRSA fecal screening prior to surgery is not performed routinely as in our institution. Identification of all MRSA reservoirs within the hospital wards is considered a major component of all control programs[10]. In this study, gastrointestinal carriage of MRSA among outpatients was similar to that from surgical wards and these patients have no history of previous hospitalization or antibiotic use. Furthermore, the majority of non-hospitalized patients were suffering from diarrhea or abdominal pain and their stool cultures were negative for common enteropathogens. However, an outbreak of gastroenteritis caused by community acquired toxin producing MRSA strains has been reported[9]. Given the emergence of community acquired MRSA it seems important to screen outpatients who are admitted to the hospital or those complaining of gastroenteritis with culture negative for common enteropathogens, for both nasal and fecal carriage of MRSA.

We conclude that gastrointestinal carriage of MRSA was higher than that of ESBL-producing organisms in our study, however, both have an important clinical implications . Therefore, fecal screening for MRSA and ESBL- producing enterobacteria is recommended for all patients at high risk admitted to the hospital wards and ICUs as well as the imple-

mentation of infection control measures to prevent dissemination of these organisms to other patients and to the community. Since our sample size was small, further studies on larger sample size are warranted in this field.

REFERENCES

1 **Gerber SI**, Jones RC, Scott MV, Price JS, Dworkin MS, Filippell MB, et al. Management of outbreaks of methicillin-resistant S. aureus infection in the neonatal intensive care unit: a consensus statement. *Infect Control Hosp Epidemiol*. 2006; 27:139-145.

2 **Pagani L**, Dell´Amico E, Migliavacca R, D´Andrea MM, Giacobone E, Amicosante G, et al. Multiple CTX-M type extended-spectrum β-lactamases in nosocomial isolates of Enterobacteriacae from a hospital in northern Italy. *J Clin Microbiol* . 2003; 41: 4264-4269.

3 **Paterson DL**, Bonomo RA. Extended-spectrum β-lactamases: a clinical update. *Clin Microbio Rev* . 2005; 18: 657-686.

4 **Babay HA.** Detection of extended-spectrum β-lactamases in members of the family Enterobacteriacae at a teaching hospital, Riyadh, Saudi Arabia. *Saudi Med J*. 2002; 23: 186-190.

5 **Rodriguez-Bano J**, Navarro MD, Romero L, Martinez-Martinez L, Muniain MA, Perea EJ, Perez-Cano R, Pascual A. Epidemiology and clinical features of infections caused by extended-spectrum beta-lactamase -producing E. coli in nonhospitalized patients. *J Clin Microbiol* . 2004; 42: 1089-1094.

6 **Maltezou HC**, Giamarellou H. Community -acquired methicillin-resistant S. aureus infections. *Int J Antimicrob Agents* . 2006; 27: 87-96.

7 **Arpin C**, Dubois V, Maugein J, Jullin J, Dutilh B, Brochet JP, et al. Clinical and molecular analysis of extended -spectrum β-lactamase-producing enterobacteria in the community setting. *J Clin Microbiol* . 2005; 43: 5048-5054.

8 **Kader AA**, Kumar A, Kamath KA. Fecal carriage of extended-spectrum β-lactamase-producing *E. coli* and K. pneumoniae in patients and asymptomatic healthy individuals. *Infect Control Hosp Epidemiol*. 2007; 28: 1114-1116.

9 **Jones TF**, Kellum ME, Porter SS, Bell M, Schaffner W. An outbreak of community-acquired foodborne illness caused by methicillin-resistant S. aureus. *Emerg Infect Dis*. 2002; 8: 82-84.

10 **Minary-Dohen P**, Bailly P, Bertrand X, Talon D. Methicillin-resistant S. aureus (MRSA) in rehabilitation and chronic-care-facilities: what is the best strategy? *BMC Geriatrics*. 2003; 3:5. This article is available from: http://www. biomedcentral. com.

11 **Valverde A**, Coque TM, Moreno-Sanchez MP, Rollan A, Baquero F, Canton R. Dramatic increase in prevalence of fecal carriage of extended -spectrum β-lactamase-producing Enterobacteriacae during nonoutbreak situation in Spain. *J Clin Microbiol* . 2004; 42: 4769-4775.

12 **Lucet JC**, Chevert S, Decre D, Vanjak D, Macrez A, Bedos JP, et al. Outbreak of multiply resistant Enterobacteriacae in an intensive care unit: epidemiology and risk factors for acquisition. *Clin Infec Dis* . 1996; 22: 430-436.

13 **Pena C**, Pujol M, Ardanuy C, Ricart A, Pallares R, Linares J, et al. Epidemiology and successful control of a large outbreak due to K. pneumoniae producing extended beta-lactamases. *Antimicrob Agents Chemother* . 1998; 42: 53-58.

14 **Jarlier V**, Nicolas MH, Fournier G, Philippon A. Extended broad-spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in Enterobacteriacae: hospital prevalence and susceptibility patterns. *Rev Infect Dis* . 1988; 10:867-878.

15 Clinical laboratory standard institute (CLSI). Performance standards for antimicrobial susceptibility testing 2005. CLSI approved standard. M100- S15. Clinical and Laboratory Standards Institute, Wayne, PA.

16 **Mirellis B**, Navarro F, Miro E, Mesa RJ, Coll P, Prats, G. Community transmission of extended-spectrum β-lactamases . *Emerg Infect Dis* . 2003; 9: 1024-1025.

17 **Filius PMG**, Gyssens IC, Kershof IM , Roovers PJE, Ott A, Vulto AG , et al . Colonization and resistance dynamics of Gram-negative bacteria in patients during and after hospitalization. *Antimicrob Agents Chemother* . 2005; 49: 2879-2886.

18 **Leistevuo T**, Toivonen P, Osterblad M, Kuistila M, Kahra A, Lehtonen AM, et al. Problem of antimicrobial resistance of fecal aerobic Gram-negative bacilli in the elderly. *Antimicrob Agents Chemother* . 1996; 40: 2399-2403.

19 **Bruinsma N**, Filius PMG, Van Den, Bogaard Nys S, Degener J, Endtz H Ph ,et al . Hospitalization, a risk factor for antibiotic-resistant E. coli in the community? *J Antimicrob Chemother* . 2003; 51:1029-1032.

20 **Johnson JR**, Sannes MR, Croy C, Johnston B, Clabots C, Kuskowski MA, et al. Antimicrobial drug resistant E. coli from humans and poultry products, Minnesota and Wisconsin, 2002-2004. *Emerg Infect Dis* . 2007; 13: 838-846.

21 **Pitout JDD**, Gregson DB, Church DL, Laupland KB. Population-based laboratory surveillance for AmpC β-lactamse-producing E. coli, Calgary. *Emerg Infect Dis* . 2007; 13: 443-448.

22 **Dupeyron C**, Campillo B, Mangeney N, Bordes M, Richardet JP, et al . Carriage of S. aureus and of Gram-negative bacilli resistant to third -generation cephalosporins in cirrhotic patients: a prospective assessment of hospital -acquired infections. *Control Hosp Epidemiol* . 2001; 22: 427-432.

23 **Rimland D**, Roberson B. Gastrointestinal carriage of methicillin-resistant S. aureus. *J Clin Microbiol* . 1986; 24: 137-138.

24 **Boyce JM**, Havill NL, Maria B. Frequency and possible infection control implications of gastrointestinal colonization with methcillin-resistant S. aureus. *J Clin Microbiol* . 2005; 43: 5992-5995.