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Original Article

Detection of resistance gene marker *intl1* and antimicrobial resistance pattern of *E. coli* isolated from surgical site wound infection in Holy Family Red Crescent Medical College Hospital

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Abstract

This study was carried out to determine the presence of resistance gene *intl1* in *E. coli* isolated from surgical site wound infection in Holy Family Red Crescent Medical College Hospital. A total of 90 samples were collected from the different wound sites of patients admitted in surgical ward. The most predominant organism was *E. coli* 48(53.33%); followed by *S. aureus* 23(25.56%) and *Pseudomonas* 12(13.33%). Most of the isolated strains of *E. coli* were multidrug resistant. 46(96%) strains were shown resistant to ampicillin and most potent drug against *E. coli* was found to be imepenem. The extended spectrum Beta-lactamase (ESBL) producing *E. coli* were 21(43.75%).The resistance gene marker *intl1* was detected in 32(67%) *E. coli* isolates.

Key words: Multidrug resistance, *E. coli, intl1*

Introduction

Surgical site infection constitutes clinical diseased states in which the multiplication of microbes in wound provokes local and systemic reactions. It has been reported that 3-11% of surgical incision became infected. Wound pathogens entered during the surgical procedure are the normal flora of the viscous[1]. On the basis of degree of microbial contamination, surgical site infections can be classified into four major groups: Clean site wounds, clean contaminated wounds, contaminated wounds, dirty or infected wounds[2]. Previous studies showed that *E. coli* and *K. pneumoniae* were identified as predominant pathogens associated with wound infection. They are the prime agents in surgical and other nosocomial (hospital-acquired) infections (wound, secondary pneumonia, peritonitis) in compromised patients[2,3].

Antibiotic resistance of *E. coli* has become a major clinical concern world wide including Bangladesh. Recently, the use of second and third generation cephalosporins has led to the selection of Gram-negative organisms like *E. coli* resistance to Beta-lactamase (ESBL) stable cephalosporins. This resistance is attributed to the production of Extended sprectum Beta lactamases. Extended spectrum Beta-lactamases are enzymes that mediate resistance to extended spectrum (third generation) cephalosporins i.e. ceftazidime, ceftriazone, cefotaxime and monobactams, i.e, aztreonam but do not affect cephamycin i.e. cefoxitin and of carbepenems i.e. meropenem or imepenem[4]. Most ESBLs are derived of TEM or SHV enzymes. With both of these groups of enzymes, a few point mutations at selected loci within the gene give rise to the extended spectrum phenotype.TEM and SHV type ESBLs are most often found in *E. coli*. All of these enzymes are closely incorporated with *intl-1* gene[5].

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Class 1 integrons are most commonly found in nosocomial and community environments followed by class 2 integrons and other integrons classes being scarcely reported to date. ESBL located on integrons like structures being increasingly reported worldwide[6,7]. The class 1 integron has been identified as the most prevalent of the five classes of integrons in clinical isolates and has been associated with multidrug resistance (MDR) in pathogenic bacteria. The class 1 integron acquires resistance genes in the form of gene cassettes via site-specific recombination. Among the same gene cassettes, some nucleotide changes tend to be present and some of these are shared by other bacteria. Certain of those nucleotide changes can be classified as singlenucleotide polymorphisms (SNPs), and these have proven useful in the fine differentiation of bacterial isolates, as well as in studies of the molecular evolution of class 1 integrons[8].

The objective of this study is to detect the resistance gene marker *intl1* in multi drug resistance *E. coli* isolated from surgical site wound infection.

Materials and Methods

A total of 90 samples were collected from the different wound sites of patients admitted in the surgical ward of Holy Family Red Crescent Medical College Hospital from July 2009 to December 2009. Duplicate wound swabs were collected; one for preparation of smear for microscopy and other for seeding of culture. Sterile cotton tipped swab was used for collecting the sample by using zig-zag motion to swab wound surface and rotating the swab during collection. Special attention was given to avoid contact surrounding skin. All specimens were inoculated into Blood agar (BA), MacConkey Agar (MCA) and Mannitol Salt Agar (MSA) media and incubated at 37^0C overnight. Bacterial isolates were identified by colony morphology, staining and appropriate biochemical tests. Isolates of *E. coli* were put into antibiotic susceptibility test by Kibry-Bauer disk diffusion technique, using panel of antibiotics as per recommendation of National Committee for Clinical Laboratory Standards, USA[9]. Screening of ESBL producing E. coli by double disc synergy test

Synergy between a disc of augmentin (amoxycillin and clavulanic acid) and 3rd generation cephalosporins was detected by double disc synergy test. The clavulanic acid in augmentin disc diffuses through the agar and inhibits the Beta –Lactamase surrounding 3rd generation cepholosporin disc. Muller Hilton Agar plates were prepared and inoculated with standardized inoculum (corresponding to 0.5 McFarland tube) with sterile cotton swab. Augmentin (20µg amoxycillin and 10µg of clavulanic acid) disc was placed in the center of the plate. The third Generation cephalosporin- ceftazidime, ceftriazone, cefotaxime and aztreonam disc was placed 20-30 mm distance from augmentin disc. The plate was incubated overnight at 37^0C. ESBL production was found to be positive when the inhibition zone around the tested antibiotic disc increased towards the augmentin disc or if neither disc was inhibitory alone but the bacterial growth was inhibited where two antibiotics diffused together[10].

Polymerase chain reaction (PCR) : A single colony from MacConkey agar plate was inoculated to 3ml of LB broth and incubated at 37°c with agitation (120 rpm).Then 1.5 ml of sample was taken in an Eppendorf tube. The sample was centrifuged at 13000 rpm for 10 min .The supernatant was discarded. The pellet was dissolved in the sterile normal saline. The sample was centrifuged at 13000 rpm for 10 min. The supernatant was discarded. The pellet was dissolved in 200µl of normal saline. The sample was boiled for 10 min. The sample was cooled in ice for 30 min. The sample was centrifuged at 13000 rpm for 10 min. The supernatant was transferred to a fresh Eppendorf tube and stored at -20°c to use template DNA in PCR. 25µl of reaction mixer containing 2.5 µl of PCR buffer(10X) 1.5 il of 50Mm $MgCl_2$) 2.0 µl Mm dNTPs, 1 µl of primer (forward and reverse) together with 0.1 unit of Taq DNA polymerase was mix gently by pippeting. Then 22 µl of reaction was taken in each PCR tube and then also took 3 µl of DNA templates from sample .Spinned for a few seconds and added one drop of PCR oil in each tube. PCR assays were performed in a DNA thermal cycle(model 480, Perkin-elmer Cetus, Emerville, USA). Each PCR tubes used the same basic setup: denaturation at 94^0C for 1min, annealing at 60^0C for 1min 15 seconds & Extension at 72^0C for1min 45 seconds. After putting all the samples in the machine, the temperature was increased at 94^0C. Finally aliquots of PCR products were analyzed by agarose (1.8%, wt/vol) gel electrophoresis in 0.5X Tris-borate-EDTA buffer, stained in ethidium bromide and visualized with a Fluoro-S MultiImager (Bio-Rad, Inc.). Primers used in the study[11]:

Target gene	Sequence (5'-3')	Amp (size)
intl1	in F: GGC ATC CAA GCA GCA AGC	600bp
	in B: AAG CAG ACT TGA CCT GAT	

Results

A total of 90 samples were collected from the different wound sites of patients admitted in surgical ward in Holy Family Red Crescent Medical College Hospital. *E. coli* (53.33%) was the predominant organisms followed by *S. aureus* (25.56%) and

Pseudomonas (13.33%). The prevalence of *Proteus* (4.44%), *Klebsiella* (2.23%) and *Streptococcus pyogens* (1.11%) were very low. (Table I)

Table-I: Rate of isolation of bacteria from surgical site wound samples (n=90)

Organisms	No. of organisms (%)
E. coli	48 (53.33)
S. aureus	23 (25.56)
Pseudomonas	12 (13.33)
Proteus	04 (4.44)
Klebsiella	02 (2.23)
Streptococcus pyogens	01 (1.11)
Acinetobacter spp.	00 (0.00)

Most of the isolated strains of *E. coli* were multidrug resistance. More than 75% strains of E. *coli* were found to be resistance to cefalexin, co-trimoxazole, nalidixic acid and tetracycline and 96% strains were shown resistance to ampicillin. The most potent drug against *E. coli* was imipenem; i.e. 100% sensitive. The result of the resistance pattern of isolated *E. coli* is shown in Table II:

Table-II: Resistance pattern of isolated *E. coli* (n=48)

Antibiotics	Resistant isolates(%)
Ampicillin	46(96)
Nalidixic acid	40(83)
Tetracycline	37(77)
Ciprofloxacin	30(62)
Ceftazidime	32(66)
Ceftriaxone	28(58)
Chloramphenicol	24(50)
Gentamicin	26(54)
Nitrofurantoin	20(42)
Co-trimoxazole	36(75)
Cephalexin	38(79)
Imipenem	00(00)

These forty Eight multidrug resistant *E. coli* strains were examined for ESBL production by double disc diffusion method. Out of those 48 *E. coli*, 21 were found to be ESBL producing. (Table III)

Table-II: Detection of ESBL positive *E. coli* strains by double disc diffusion method (n=48)

Organisms	No. of organisms (%)
ESBL-positive strains of *E. coli*	21 (43.75)
ESBL –negaive strains of *E.coli*	27 (56.25)

All forty eight *E. coli* isolates were examined for resistance gene marker *intl1* and out of which 32 isolates were positive for *intl1* (67%).The presence of integrons was explored by PCR to identify class I integron which are specific for the 5' conserved segment containing integrase gene (*intI*-1). In this study, PCR within Int-U and Int-D primers yielded amplicon for 32 of the 48 strains tested including the desired amplicon for the positive control. (Figure I)

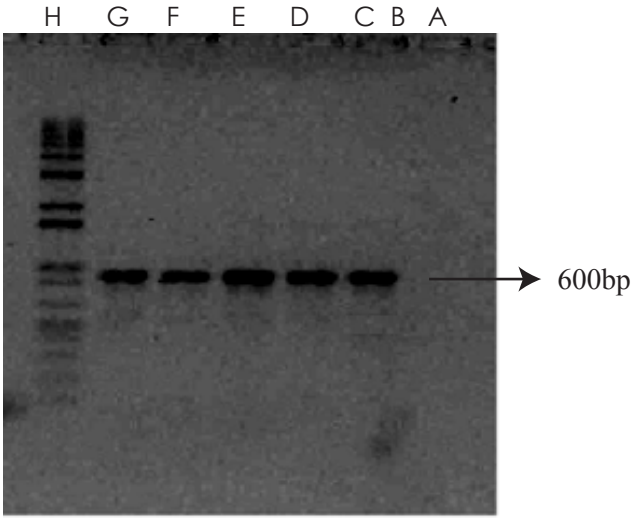

Figure:I Agarose gel electrophoresis showing PCR amplification products of the *intl1*.Lane D E F G representative of *E.coli* E-19, E-10, E14, E-15. Lane B negative control. Lane C positive control, Lane A reagent blant and Lane H DNA molecular size marker(1kg DNA ladder from GIBCO- BRL).

Discussion

Considering the strains of Gram negative bacteria, *E. coli* was the most frequently isolated organism from wound infection. Higher prevalence of *E. coli* in our study might be due to its frequent presence in hospital environment from where study cases were selected and in most surgical site wounds *E. coli* is usually the predominant organism[12]. In this study, the rate of *Pseudomonas* in wound infection is 12%. A lower rate of *Pseudomonas* has also been reported by and Mahmood[13] from Pakistan (13%); but Shasuzzaman *et al*[14] from Bangladesh

has reported higher rate of Pseudomonas 37.2%. Other organisms in order of frequency of isolation included *Proteus* (4.44%), *Klebsiella* (2.23%); almost similar result has been reported by Shittu *et al*[15]. The rate of infection by *Streptococcus pyogenes* in this study has been observed to be quite low; similar result was reported by Jinnah *et al*[16] from Bangladesh (3.1%). This static and low rate of infection by *Streptococcus pyogenes* in different studies may be due to the fact that penicillin has always been used successfully for combating infection by *Streptococcus pyogenes.* Among the Gram positive organisms, the presence of *S. aureus* with their resistant strain MRSA is very high, which was stated in another study[17].

Resistance pattern of antibiotics to microbial flora of surgical site infection revealed in the present study that most of the *E. coli* isolates were resistance to at least 3 drugs and maximum isolates were resistance to 6 to 7 drugs whereas some of them were found to be resistance to all drugs. Similarly, Shamsuzzaman *et al* [18] observed a gradual increase in resistance pattern of *E. coli* in different years against almost all the antibiotics except imipenem and pefloxacin. The presence of ESBL producing *E. coli* stains in this study is 43.75%. In Bangladesh, a study by Rahman *et al* [4] showed 43.21% *E. coli* ESBL producer and Alim *et al*[19] found 41.39% ESBL producing organisms. So, the rates of ESBL producing *E. coli* in these three studies in Bangladesh are almost similar.

Since antibiotic resistance is a major phenotypic trait particularly for the clinical isolates, it has a potential interest in exploring the characteristic of these ESBL producing isolates of *E. coli*. In the present study, most ESBL producers collected from patients in the post operative room and surgical ward. These patients were exposed to great antibiotic pressure. The susceptibility test results showed that all the ESBL producing isolates were resistant to 3rd generation Cephalosporin, This reflected the relationship between the ESBL and to 3rd generation cephalosporins. Increased resistance might be due to extensive use of 3rd generation cephalosporins and other Beta- lactum drugs. This finding justifies that ESBLs producing *E. coli* are multidrug resistance. Infection caused by ESBL producing organisms have currently been treated with carbepenems such as imipenem[20].

Gene coding ESBLs are usually located on conjugative plasmids although many of the most recently described ESBL genes are frequently found within integron like structure[6,7]. Integrons are naturally efficient recombination and expression systems able to capture genes as part of genetic elements known as gene cassettes. Out of five integrons, Class 1 integrons are the most commonly prevalent in nosocomial and community environments. ESBL located on integrons-like structure are being increasingly reported world wide[21]. Class 1 integrons of *E. coli* is closely associated with human related environments that are more likely affected by antibiotic selective pressures. Two pressures might be at work. The presence of antibiotics selecting some genes associated to integrons, such as Sulfonamide resistance genes or any mobile cassette acting as a facilitator and promoting their retention. As gene cassettes within *E. coli* integrons appear to be mostly related to antibiotic resistance traits, it can be presumed that antibiotics exert the main pressure that selects and or maintains integrons in *E. coli.* Together this indicates that lifting the selection pressure posed by antibiotics might not reduce the prevalence of resistance itself, but instead that of some of the mobile genetic elements by which resistance can be transferred[11]. The second possibility is that the sparse distribution of the *intl1* PCR positive isolates among non human *E. coli* strains might be a consequence of the lesser ability of those strains to acquire integrons, or simply that do not interact with integron bearing organisms rather than a higher loss rate. Furthermore, integrons have been found in a wide variety of environments with and without human influences, so that these elements are readily available for non human strains[11].

E. coli may collect certain intregron elements that can exist without much need of antibiotic selection and high prevalance of integron elements has a cumulative effect. Although there is a chance of finding the presence of intregron elements in the absence of antibiotic selection pressure, the high percent of integron intregase (67%) observed in this study indicates there has already a strong selection pressure in the community[22]. Different studies have suggested that integrons are directly responsible for resistance to trimethoprim and aminoglycosides and the presence of integron is significantly associated with multidrug resistance in commensal *E. coli* isolates[23]; which is also observed in this study.

The result presented in this study confirms that the most commonly used antibiotics against *E. coli* of surgical site wound become increasingly resistant and it is the right time to ensure the judicial use of antibiotics. Finally, it is important to find the mobile antibiotic gene cassette of *intl1* along with their incorporated ESBL genes (TEM, SHV) to identify the

inherent drug resistance pattern of *E. coli*.

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References:

1. Nichols RL. Postoperative infections in the age of drugs- resistant gram-positive bacteria. Am J Med 1998; 29:1049 (5A) 11S-16S.

2. Anaya DA and Dellinger EP. Challenges in the prevention of surgical site infections. Infections in Medicine 2006;23:120-126

3. Bradford PA. Extended sprectum â lactamases in the 21st century: Characterization, epidemiology and detection of the important resistance threat. Clin Microbiol Rev 2001; 14:933-951.

4. Rahamn M, Haq JA, Hossain MA, sultana R, Islam F, Islam S. Prevalence of extended-spectrum Beta lactamase producing *Escherichia coli* and *Klebsiella pneumonia* in an urban hospital in Dhaka, Bangaldesh. Int J antimicrob agents 2004;24:508-10

5. Bush K, Jacoby GA and Medeiros AA. A functional classification scheme for â-lactamases and its correlation with molecular structure. Antimicrob. Agents. Chemother 1955; 39:1211-1233.

6. Canton R, Coque TM and Baqueero F. Multi resistance gram-negative bacilli: from epidemics to endemics. *Curr Open Infect*. Dis 2003;16: 315-325.

7. Bonnnet R. Growing group of extended spectrum Beta-lactamases: The CTX-M enzymes. Antimicrob Agents Chemother 2004;48:1-14.

8. Sokurenko, E. V., D. L. Hasty, D. L. Dykhuizen. Pathoadaptive mutations: gene loss and variation in bacterial pathogens. Trends Microbiol 1999;7:191–195.

9. National Committee for Clinical Laboratory Standards. Performance standard for antimicrobial susceptibility testing. Suppliment M100-S9. NCCLS, Wayne, PA.1999.

10. Jarlier V, Nicolas M, Fournier G, Phillippon A. ESBL conferring transferable resistance to newer â-lactam agents in Enterobacteriaceae: hospital preventable and susceptibility patterns. Rev. Infect. Dis 1988 ;10:867-878

11. Diaz-Mejia JJ, Carlos F, Amabile-Cuevas CF, Rosas I, Souza V. An analysis of the evolutionary relationship of integron integrases with emphasis on the prevalence of class1 integrons in *E. coli isolates* from clinical and environmental origins. Microbiology 2008;154**:**94-102.

12. Zafar A. Prevalent nosocomial gram negative aerobic bacilli and their antimicrobial susceptibility pattern in intensive care unit. J Pak Med assoc 1999;49(7):169-172.

13. Mahmood A. Bacteriology of surgical site infections and antibiotic susceptibility of the isolates at a tertiary hospital in Karachi. J Pak Med Assoc. 2000;50(8):256-259.

14. Shamsuzzaman AK, Sirajee A, Rahman M, Miah AG, Hossain MA. Pattern of aerobic bacteria with their drug susceptibility of surgery in patient. Mymensingh Med J. 2003;12(2)**:**98-103 .

15. Shittu AO, Kolawole DO and Oyedepo EA. A study of wound infection in two health institutes in IIe-Ife, Nigeria. Afr J Biomed Res 2003;5:97-102.

16. Jinnah, F, Chowdhury K, Begum J Sohali M, Rahman MT, Ahmed S, Rumi MAK, Morshed MG, Huq F.. Multi resistant Staphylococcus aureus isolated from wound of diabetic patient. J Infect Dis antimicrob agents. 1998;15:15-18.

17. Murshed M, Kamar S, Malek MA, Haque M, Akhter N. A comparative study of surgical site wound infection in DMCH and HFRCMCH and detection of MRSA. OMTAJ 2011;10(1):36-41.

18. Shamsuzamman AKM, Paul SK, Mahmud MC, Musa AKM, hossain MA. Emerging antimicrobial resistance amongst common bacterialpathogens in Mymensingh Medical College Hospital. Bangladesh J Med Microbiol 2007;01: 04-09

19. Alim R, Miah RA, Saleh AA, Bari MS. ESBL producing bacteria in urban hospital of Bangladesh. Bangladesh J Med Microbiol 2009;01:04-09

20. Pitout JDD, Thomson KS, Hanson ND, Ehrhardt AF, Moland ES, Sanders CC. Beta-lactamases responsible for resistance to extended spectrum cephalosporins in *Klebsiella pneumoniae*, *Escherichia coli* and *Proteus mirabilas* isolated recovered in South Africa. Antimicrob Agents Chemother 1998; 42:1350-1354

21. Rowe-Magnus DA and Mazel D. The role of integrons in antibiotic resistance gene capture. Int J Med Microbiol 2002; 292:115-125

22. Pongpech P, Naenna P, Taipobsakul Y, Tribuddharat C, Srifuengfung S. Prevalence of ESBL and Class 1 integron intregase gene *intl1* in *E.coli* from Thai patients and healthy adults. South A J Trop Med Public Health 2008;39(3):425-433

23. Lee JC, Kang HY, Oh JY, Jeong JH, Seol SY, Cho DT, Kim J, Lee YC.2006. Antimicrobial resistance and integrons found in commensal *E.coli* isolated from healthy humans. Journal of Bacteriology and virology 2006;36(3):133-139