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Molecular Study of *E. coli* Virulence genes in Nosocomial Sepsis

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Escherichia coli (E. coli) **is a common cause of nosocomial sepsis. There are multiple factors related to the severity of sepsis among these are the presence of virulence genes and the pattern of antibiotics resistance. The aim of the present study was to determine the prevalence of virulence pap gene encoding for pili, *hlyA* gene encoding for á-hemolysin and *cnf1* gene encoding for cytotoxic necrotizing factor 1 among *E.coli* isolated from children with nosocomial sepsis. Also, to correlate the presence of ESBL and carbapenem resistance with the presence of these genes. The study is a retrospective cross-sectional study included 150 non-duplicate strains of *E.coli* isolated from blood cultures from children with nosocomial sepsis. The isolated *E.coli* strains were subjected to antibiotics study by disc diffusion method, detection of extended spectrum lactamase production by double discs diffusion method and determination of resistance to carbapenem by combined tests methods. The detection of virulence genes *pap, hylA*and *cnf-1* were determined by multiplex polymerase chain reaction (PCR). *E.coli* isolates were classified as ESBL phenotype in 56% of the isolates and carbapenemase producing phenotype in 34.7%. *Pap* gene, *hylA* and cnf-1 genes were detected in 30%, 23.3% and 22.7% of the isolated *E.coli.* The clinic-laboratory study of the virulence genes of *E.coli* revealed the significant association of *pap*, *hylA* and *cnf-1*genes with prolonged duration of the use of the medical devices (4.3± 2.9 days-P=0.01, 4.5± 2.9 days, P=0.02, 5.2± 3.4 days, P=0.0001 respectively).*HylA* gene was associated with younger age of the patients (28.4± 4.5, P=0.01). *Pap* gene was significantly associated with ESBLs and carbapenemase phenotypes (P=0.0001, P=0.002 respectively). On the other hand,*cnf-1* was significantly associated with *E. coli* isolated from primary sepsis (P=0.02) and in isolates from sepsis due to medical devices (P=0.02) and was significantly associated with death (P=0.01) and carbapenemase resistance (P=0.01). The present study highlights the prevalence of *pap, hylA* and *cnf-1* virulence genes among *E. coli* associated with nosocomial sepsis in children. The frequency of some of these genes was correlated with extended spectrum lactamase resistance and carbapenemase resistance. This may be attributed to the presence of the virulence and antibiotics genes on transferable plasmids. Moreover, there was association with *cnf-1* virulence gene and mortality outcome of sepsis. Further studies are recommended to evaluate these findings.**

Keywords: *E. coli, pap, hylA, cnf-1*, multiplex PCR.

Sepsis is a serious infection affecting children admitted to intensive care units. There are various pathogens associated with this infection either gram negative bacilli, gram positive cocci and fungal pathogens. *Escherichia coli*(*E. coli*) represents a major etiology of sepsis[1,2]. Sepsis due to *E. coli* can result as a complication of infections of urinary tract or gastrointestinal tract. It may also

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arise as a primary blood stream infection without an obvious source[3].

Various factors are correlated with the severity of *E. coli* associated with sepsis. Among these factors are resistance pattern to antibiotics that has emerged among *E. coli*. The isolated *E. coli* from sepsis has been shown to produce Extended spectrum â-lactamases (ESBLs)[4, 5]. Another threat of antibiotics resistance among clinical isolates of *E. coli* is the resistance to carbapenem antibiotics namely to meropenem and/or imipenem[6]. The risks factors for acquiring nosocomial sepsis due to ESBLs strains of *E. coli* and carbapenem resistant strains include the duration of intensive care units stay, age of the patients, presence of co morbidities and associated devices[4,7].

Other factor that determines the severity of sepsis due to *E. coli* is the presence of the virulence genes. Virulence genes of *E. coli* act through several mechanisms. First mechanism is associated with invasion of blood stream controlled by genes that encode for amyloid curli and gene of siderophores that support survival outside gastrointestinal tract[8]. Second mechanism is by facilitating the attachment of *E. coli* to cells by the means of fimbria factors P and S and causingdamage to these cells by hemolysin and cytotoxin necrotizing factors[9, 10]. Third mechanism is associated with the presence of capsular K1 that inhibits phagocytosis and complement mediated-killing[11,12]. The fourth mechanism is by the production of bacteriocins by certain *E. coli* strains[13].

The aim of the present study was to determine the prevalence of virulence pap gene encoding for pili,*hlyA* gene encoding for á-hemolysin and *cnf1* gene encoding for cytotoxic necrotizing factor 1 among *E. coli* isolated from children with nosocomial sepsis. Also, to correlate the presence of ESBLs and carbapenem resistance with the presence of these genes.

MATERIAL AND METHOD

The study is retrospective cross-sectional study included 150 non-duplicate strains of *E. coli* isolated from blood cultures from children with nosocomial sepsis admitted to ICUs in Mansoura University Children hospital, Egypt from January 2016 till January 2018. The study was approved by Mansoura Faculty of Medicine ethical committee and approval consent was obtained from the parents of each child.

The clinical data of each participating child was obtained as regard age, sex, the presence of comorbidities conditions, underlying medical conditions, the presence of central venous line, urinary catheter and other devices and the outcome of infection after 30 days was recorded either death or discharge. The bacteremia was defined as secondary if the cause of sepsis was identified according to clinical and/or laboratory evidence of infection and primary if there was no evidence of the infection

Positive blood culture from defined nosocomial sepsis which is defined as the infection that occurred after 48hours from hospital admission according to CDC definitions was processed according to the standard microbiological methods. Blood culture was obtained under complete sterile conditions and inoculated to blood culture bottles Bact/alert system. Positive blood culture was processed by subculture on blood agar plates and incubated at 37°C for 24 hours. The culture was identified by gram stain followed by biochemical identification by the use of automated microbiology system Microscan (WalkAway 40 plus System- B1018-283- Beckman Coulter, Inc). Antimicrobial susceptibility testing was performed by the use of the Kirby–Bauer disc diffusion method. Production of ESBLs was confirmed using the double-disk synergy test in accordance with the Clinical and Laboratory Standards Institute standards[14]. Carbapenem producing *E. coli* strains were defined as resistant strains to imipenem and/or meropenem discs and confirmed by combined discs diffusion method by the use of EDTA and boronic acid.

Antibiotics Discs diffusion

The used antibiotics discs were ceftazidime (30 µg), cefotaxime (30 µg), cefepime (30 µg), imipenem (10 µg), meropenem (10 µg), gentamicin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), sulfamethoxazole/trimethoprim(1.25/23.75 µg), and piperacillin/tazobactam (100 µg/10 µg) (Oxoid--Thermo Fisher Scientific-USA 02451). The interpretation of the results was done according to the Clinical and Laboratory Standards Institute (CLSI) guidelines[14].

Double Discs Diffusion Method (DDT) for ESBLs

Resistant strains of *E. coli* for ceftazidime and cefotaxime antibiotics discs were used for confirmatory phenotypic tests for EDBLs production by DDT. The test depends upon increase the inhibition diameter zone around each disc when antibiotics were conjugated with clavulanic acid a known inhibitory for extended spectrum â-lactamase enzyme by ≤5 mm. The test was performed as previously described[14].

Determination of carbapenemases production by combined disk test (CDT) and boronic acid discs

E. coli strains with resistance to imipenem and/or to meropenem were subjected to determination of carbapenemases by CDT and boronic acid discs amino phenylboronic acid (APBA). The positive CDT detects the possible production of metallo-â-lactamase (MBL) and the positive boronic acid disc detects the possible presence of class A carbapenemase (14). The increaseof the inhibitory zone around carbapenem discs by ≤5 mm when combined â-lactamase inhibitor (APBA or EDTA) was considered to be a positive result[16].

Quality Control

E. coli ATCC 25922 was used as a susceptible strain for antibiotics susceptibility tests. ESBL-positive *Klebsiellapneumoniae* ATCC 700603 and ESBL-negative *E. coli*ATCC 25922 control strains were used in this study. For carbapenemase detection, *E. coli* ATCC 25922 strain was used as a negative control.

Molecular Study of *E. coli*

Extraction of *E. coli* DNA

E. coli was subculture on nutrient agar at 37°C for 24 hours. The colonies were obtained and suspended in Tris-EDTA buffer (10 mMTris-HCI and 1 mM EDTA) and subjected to centrifugation at 2000 g for 10 min. The supernatant was removed and the pellet was incubated with proteinase K and Tris-EDTA buffer for 18 hours at 55° C in water bath. Then, the DNA was extracted by phenol and chloroform extraction method and suspended in Tris-EDTA buffer and kept frozen at -20°C for polymerase chain reaction (PCR)[15].

PCR for Identification of *E. coli*

The isolates were confirmed as *E. coli* by detection of gene of*Enterobacteriaceae* common antigen (ECA) specific for E. coli. PCR method. The used specific primers for *E. coli* were listed in table 1[15]. The amplification was performed by the use of Qiagen amplification mixture with total volume 50lμ (Qiagen-Germany). The amplification procedures were initial denaturation at 95° C for 7 min; followed by 35 cycles of denaturation at 95° C for 1.5 min, annealing at 65° C for1.5 min and extension at 72° C for 1.5 min and a final extension for 7 min at 72° C. Ten microliters of the reaction mixture was analyzed on 1.5% agarose gel by electrophoresis. The bands were visualized by ethidium bromide staining under UV.

Multiplex PCR for Detection of Virulence Genes

The multiplex PCR was performed by the use of ready to use Qiagen amplification mixture with total volume 50 µl. The sequences of the used primers were summarized in table 1. The amplification procedures were initial denaturation at 94° C for 3 min, followed by 30 cycles of denaturation at 94° C for 1 min, annealing at 63° C for 30 s and extension at 72° C for 3 minutes and the final extension for 7 minutes at 72° C.

A volume of 10 ml of the reaction mixture was analyzed on 2% agarose gel by electrophoresis. Bands were visualized under UV after ethidium bromide staining[15].

Statistical Analysis

Data were analyzed by the use of statistical package for social science (SPSS) version 24. The quantitative data were presented as mean, standard deviations and ranges. The qualitative data were presented as percentage and compared by the use of chi-square. The comparison between the studied groups were done by using One Way Analysis of Variance (ANOVA). P was considered significant if d"0.05. Multiple logistic regression was performed by the use of binary model.

RESULTS

The study included non-replicated isolates of *E. coli*. The isolates were from children with nosocomial sepsis with median age 6.7 months and they were mainly males 53.3%. The sepsis was primary sepsis in 83.3% of the patients associated with devices central venous catheter, urinary catheter and ventilator (64%). The mortality among these series of patients was 31.3%. *E. coli* isolates had ESBLs phenotype in 56% of the isolates and

carbapenemase producing phenotype in 34.7%. *Pap* gene, *hylA* and cnf-1 genes were detected in 30%, 23.3% and 22.7% of the isolated *E. coli*, table 2.

The clinic-laboratory study of the virulence genes of *E. coli* revealed the significant association of *pap*, *hylA* and *cnf-1* genes with prolonged duration of the use of the medical devices (4.3± 2.9 days-P=0.01, 4.5± 2.9 days, P=0.02, 5.2± 3.4 days, P=0.0001 respectively).*HylA* gene was associated with younger age of the patients (28.4±4.5, P=0.01). *Pap* gene was significantly associated with ESBLs and carbapenemase phenotypes (P=0.0001, P=0.002 respectively). On the other hand,*cnf-1* was significantly associated with *E. coli* isolated from primary sepsis (P=0.02) and in isolates from sepsis due to medical devices (P=0.02) and was significantly associated with death (P=0.01) and carbapenemaseresistance (P=0.01), table 3.

Multiple logistic regressionanalysis of possible risk factors associated with death reveals significant association with younger age and the presence of *cnf-1* as significant risk factors (P=0.012, P=0.004 respectively), table 4

The isolated *E. coli* had high prevalence of resistance toward ceftazidime, meropenem and imipenem (73.2%, 58.7%, 53.3% respectively) with lower prevalence of resistance to cefepime, amikacin and ciprofloxacin (17.3%, 13.3%, 20% respectively), figure 1.

Table 1. Genes, primers sequences and amplification products bp

Gene	Sequences of the Primers	bp
E.col	5'-GGT GGTCGG CAA GCT TTA TCT CAGGGT-3' 5'-TAA ATT GGG GCT GCC ACC ACG-3'	793
*pap*G	5'-GCA ACA GCA ACG CTG GTT GCA TCA T-3' 5'-AGA GAG AGC CAC TCT TAT ACG GAC A-3'	336 bp
*hly*A 5'	5'-AAC AAG GAT AAG CAC TGT TCT GGC T-3' 5'-ACC ATA TAA GCG GTC ATT CCC GTC A-3'	1177 bp
*cnf*1	5'-AAG ATG GAG TTT CCT ATG CAG GAG-3' 5'-CAT TCA GAG TCC TGC CCT CAT TAT T-3'	498 bp

Table 2. Demographic, clinical and laboratory data of the studied patients

Gender		
Male	80	53.3%
Female	70	46.7%
Age (months)		
Minimum	1.00	
Maximum	192.00	
Median	6.7	
Type of Sepsis		
Primary	125	83.3%
Secondary	25	16.7%
Type of Sepsis		
Device associated	96	64%
Non-device associated	54	36%
Central venous catheter	72	48%
Urinary catheter	44	29.3%
Ventilator	24	16%
Virulence genes		
Pap	45	30%
hlyA	35	23.3%
cnf-1	34	22.7%
ESBLs	84	56%
Carbapenemase	52	34.7%
Outcome		
Death	47	31.3%
Survive	103	68.7%

DISCUSSION

E. coli has different strains that can act as a commensal in gastrointestinal tract, diarrheagenic bacteria or an extraintestinal tract pathogen. It is a common cause of nosocomial infections with presence of several virulence genes that are not present in the commensal strains[16]. Beside virulence factors associated with pathogenic *E. coli*, antibiotics resistance seems to play a vital role in its association with severe infections such nosocomial sepsis.

In the present report the prevalence of the virulence genes *pap*, *cnf-1* and *hylA*were studied in correlation with antibiotics resistance and outcome of nosocomial sepsis in children.

The frequency of *cnf-1* gene among clinical isolates of*E. coli* was 22.7%. This frequency is online with findings of previous studies with range from 14.2% up to 30%[17-19]. The frequency was less in commensal isolates. Moreover, *cnf-1* gene was the only virulent gene reported in the present study to be correlated with mortality (P=0.004). There are scarce data about

Table 3. Clinco-laboratory study of the presence of virulence genes of E. coli

	Pap(n=45)		*hlyA*(n=35)		*cnf-1*(n=34)	
Geneder						
Male	22	48.9%	20	57.1%	16	47.1%
Female	23	51.1%	15	42.9%	18	52.9%
P	0.3	0.4	0.3			
Age						
Present	55.8± 37.3	28.4± 4.5	52.2± 33.3			
Absent	52.7± 32.9	72.3± 5.4	57.5± 33.4			
P	0.1	0.01	0.9			
Type of sepsis						
Primary	35	77.8%	32	91.4%	24	70.6%
Secondary	10	22.2%	3	8.6%	10	29.4%
P	0.2	0.1	0.02			
CVC	22	48.9%	19	54.3%	16	47.1%
P	0.5	0.4	0.5			
Urinary Catheter	14	31.1%	9	25.7%	10	29.4%
P	0.8	0.4	0.5			
Device	20	44.4%	17	48.6%	21	61.8%
associated	0.1	0.1	0.04			
Duration of the presence	4.3± 2.9	4.5± 2.9	5.3± 3.4			
of the device till sepsis (mean±SD) days						
Absent	3.1±2.6	2.5±1.1	2.9±2.2			
P	0.01	0.02	0.0001			
ESBLs	42	93.3%	26	74.3%	22	64.7%
P	0.0001	0.02	0.2			
Carbapenemase	24	53.3%	12	34.3%	18	52.9%
P	0.002	0.5	0.01			
Outcome						
Death	14	31.1%	13	37.4%	30	88.2%
Life	31	68.9%	22	62.9%	4	11.8%
P	0.6	0.3	0.01			

Table 4. Multiple logistic regression study of risk factors associated with death

	B	Wald	Sig.	Exp(B)	95% C.I.	
					Lower	Upper
Age	.012	6.364	.012	1.012	1.003	1.022
Gender	.514	1.671	.196	1.671	.767	3.641
ESBLS	-.616	1.882	.170	.540	.224	1.302
EDT	.431	1.036	.309	1.539	.671	3.531
IV_Days	-.005	.004	.952	.995	.856	1.157
Device_associated	.088	.058	.810	1.092	.532	2.242
pap	.060	.013	.909	1.062	.383	2.941
hly	-.118	.053	.817	.889	.326	2.422
cnf1	-1.436	8.172	.004	.238	.089	.637

association of cnf-1 gene with outcome of sepsis in previous studies[18, 19] witha suggestionthat there is a link between fatal outcome and the presence of this gene. This link may be attributed to the fact that *cnf-1* codes for cytotoxic necrotizing factor 1 that affects the host cellular immune functions leading to inhibition of phagocytosis and chemotactic activities of neutrophils[20, 21].

The mortality rates in the present series of patients was 31.1% which is an expected rate as the sepsis was mainly primary sepsis in 83.3% of the patients associated with the use of medical devices in 64%. The mortality rates in previous reports range from 5% up to 30% in sepsis due to non-urinary *E. coli* sepsis[19, 22].

The frequency of *pap* gene was 30% with non-significant correlation with mortality outcome. Previous data supported the present finding with pap gene the most frequent gene associated with *E. coli* as it encodes for the P fimbriae that mediates an important function of bacterial adherence to host cells[23]. However, its relation to outcome of sepsis had significant variance in the studies, as one study reported an association to fatal outcome[18] while other study claimed a protective role for this gene from fatal outcome due to the immunogenicity of

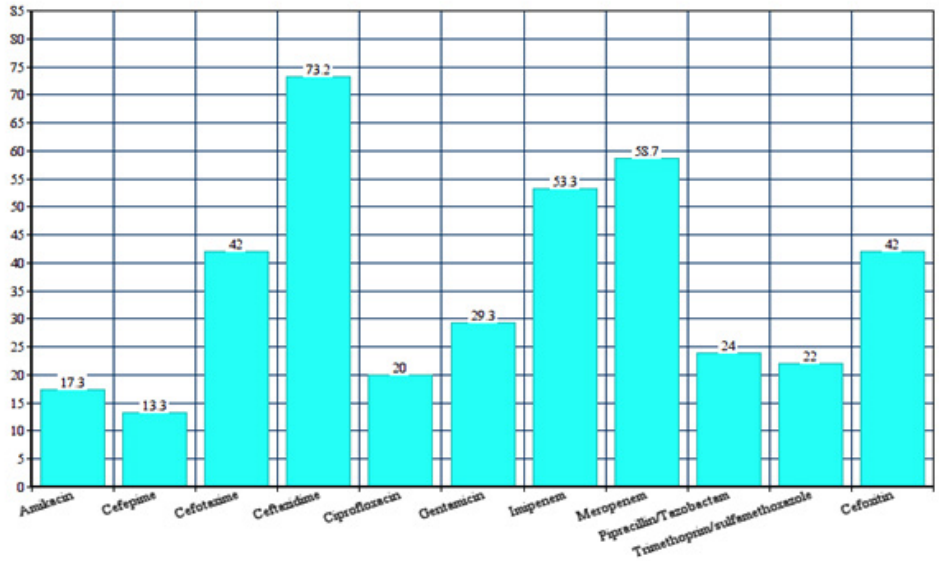
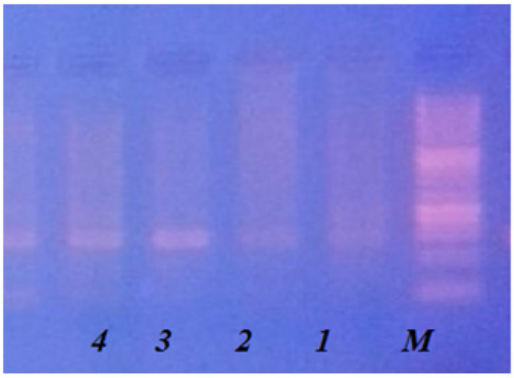


Fig. 1. Antibiotics resistance among *E. coli*



M: marker
Lanes 1-4 positive *E. coli* for *pap* gene
Fig. 2. Positive *E.coli* for*pap* genes

the P fimbriae leading to early and strong immune response that controls the sepsis[24].

The frequency of hyla gene was 23.3%. Hemolysin (*hly*) is produced by various pathogenic types of *E. coli* causing extraintestinal and intestinal infections, but its effect on virulence is not completely clarified[25,26]. *HlyA* gen region was related with complicated urinary tract infections such as pyelonephritis and cystitis[27, 28].

The frequency of detections of *pap,hylA* and *cnf-1* genes were correlated with the prolonged duration of the use of the medical devices (P=0.01, P=0.02, P=0.0001 respectively). Previous reports confirmed the correlation between device associated infections and virulence genes due to

increase capacity of *E. coli* to form biofilm difficult to treat[16, 29]. From the present study this capacity may be increased by prolonged use of medical devices such as central venous catheter, ventilator and urinary catheter

The pap gene was correlated with ESBLs and carbapenemase resistant phenotypes and *cnf-1* was correlated with carbapenemase phenotype. The correlation between virulence genes and antibiotics resistance in *E. coli* are controversy, while some studies reported this association with ESBLs (30, 31) other study reported no correlation[32]. There are many theories about the association between antibiotics resistance and virulence genes one theory attributed this association due to the presence of both virulence genes and antibiotics resistance genes on the same plasmid while the other theory related this to porin loss[33], and modifications in the penicillin binding proteins and efflux pumps mechanism[34]. Efflux pumps are responsible for the secretion of the molecules containing virulence factors that is regulated by quorum sensing which has a positive effect on antibiotics resistance and virulence[35,36].

The isolated *E. coli* had high prevalence of resistance toward ceftazidime, meropenem and imipenem with lower prevalence of resistance to cefepime, amikacin and ciprofloxacin.The high frequency of antibiotics resistance among E.coli may be attributed to the improper use of antibiotics with selective pressure leading to the prevalence of resistant strains[37]. This finding warrants the need for strict implantation of antibiotics policy.

The present study highlights the prevalence of *pap, hylA* and *cnf-1* virulence genes among *E. coli* associated with nosocomial sepsis in children. The frequency of some of these genes was correlated with extended â- spectrum lactamase resistance and carbapenemase resistance. This may be attributed to the presence of the virulence and antibiotics genes on transferable plasmids. Moreover, there was association with *cnf-1* virulence gene and mortality outcome of sepsis. Further studies are recommended to evaluate these findings.

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REFERENCES

1. Geerdes HF, Ziegler D, Lode H, Hund M, Loehr A, Fangmann W, *et al*. Septicemia in 980 patients at a university hospital in Berlin: Prospective studies during 4 selected years between 1979 and 1989. *Clin Infect Dis* 1992; **15**: 991-1002
2. Reddy, E.A., Shaw, A.V., Crump, J.A., Community-acquired bloodstream infections in Africa: a systematic review and meta-analysis. *Lancet Infect. Dis.* 2010; **10**(6); 417-432.
3. Laupland, K.B., Gregson, D.B., Church, D.L., Ross, T., Pitout, J.D., Incidence, risk factors and outcomes of Escherichia coli bloodstream infections in a large Canadian region. *Clin. Microbiol. Infect.* 2008; **14**(11): 1041-1047.
4. Denis B, Lafaurie M, Donay JL, *et al*. Prevalence, risk factors, and impact on clinical outcome of extended-spectrum beta-lactamase-producing Escherichia coli bacteraemia: a five-year study. *Int J Infect Dis* 2015; **39**: 1–6.
5. Cornejo-Juárez P, Pérez-Jiménez C, Silva-Sánchez J, *et al*. Molecular analysis and risk factors for Escherichia coli producing extended-spectrum â-lactamase bloodstream infection in hematological malignancies. *PLoS One* 2012; **7**: e35780
6. McLaughlin M, Advincula MR, Malczynski M, Qi C, Bolon M, Scheetz MC. Correlations of Antibiotic Use and Carbapenem Resistance in *Enterobacteriaceae*, *Antimicrobial Agents and Chemotherapy* 2013; **57**:5131-3.
7. Rodríguez-Baño J, Navarro MD, Romero L, *et al*. Risk-factors for emerging bloodstream infections caused by extended-spectrum beta-lactamase-producing Escherichia coli. *ClinMicrobiol Infect* 2008; **14**(2):180–3.
8. Hung, C., Marschall, J., Burnham, C.A., Byun, A.S., Henderson, J.P., The bacterial amyloid curli is associated with urinary source bloodstream infection. *PLoS One.* 2015; **9**(1): e86009.
9. Johnson, J.R., Virulence factors in Escherichia coli urinary tract infection. *Clin.Microbiol. Rev*. 1991; **4**(1), 80-128.
10. Mulvey, M.A., Adhesion and entry of uropathogenic Escherichia coli. *Cell Microbiol*. 2002; **4**(5): 257-71
11. Gaschignard, J., Levy, C., Romain, O., Cohen, R., Bingen, E., Aujard, Y., Boileau, P., Neonatal Bacterial Meningitis: 444 Cases in 7 Years. *Pediatr. Infect. Dis. J.* 2011; **30**(3): 212-217.
12. Stoll, B.J., Hansen, N.I., Sanchez, P.J., Faix, R.G., Poindexter, B.B., Van Meurs, K.P., Bizzarro, M.J., Goldberg, R.N., Frantz, I.D.III., Hale, E.C., Shankaran, S., Kennedy, K., Carlo, W.A., Watterberg, K.L., Bell, E.F., Walsh, M.C.,

Schibler, K., Laptook, A.R., Shane, A.L., Schrag, S.J., Das, A., Higgins, R.D., Early onset neonatal sepsis: The burden of group B streptococcal and E. coli disease continues. *Pediatrics.* 2011; **127**(5): 817–826.

13. Micenková, L., Bosák, J., Štaudová, B., Kohoutová, D., Èejková, D., Woznicová, V., Vrba, M., Ševèíková, A., Bureš, J., Šmajs, D., Microcin determinants are associated with B2 phylogroup of human fecal Escherichia coli isolates. *Microbiology Open*. 2016; **5**(3): 490-498.
14. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing, 24th Informational Supplement (M100-S24). Wayne, PA: Clinical and Laboratory Standards Institute; 2014:34(1). https://clsi.org/standards/products/microbiology/documents/m100/
15. Arisoy M, Aysev D, Ekim M, Ozel D, Köse SK, Ozsoy ED, Akar N. Detection of virulence factors of Escherichia coli from children by multiplex polymerase chain reaction. *Int J ClinPract.* 2006; **60**(2):170-3.
16. Naves P, del Prado G, Huelves L, Gracia M, Ruiz V, Blanco J, Dahbi G, Blanco M, Ponte Mdel C, Soriano F. Correlation between virulence factors and in vitro biofilm formation by Escherichia coli strains. *MicrobPathog.* 2008 **45**(2):86-91. doi: 10.1016/j.micpath.2008.03.003.
17. Lefort A, Panhard X, Clermont O, Woerther PL, Branger C, Mentré F, Fantin B, Wolff M, Denamur E; COLIBAFI Group. Host factors and portal of entry outweigh bacterial determinants to predict the severity of Escherichia coli bacteremia. *J Clin Microbiol* 2011; **49**: 777-83;PMID:21177892; http://dx.doi.org/10.1128/JCM.01902-10,
18. Jauréguy F, Carbonnelle E, Bonacorsi S, Clec'h C, Casassus P, Bingen E, Picard B, Nassif X, Lortholary O. Host and bacterial determinants of initial severity and outcome of Escherichia coli sepsis. *Clin Microbiol Infect* 2007; **13**: 854-62; PMID:17617183
19. Mora-Rillo M, Fernández-Romero N, Francisco CN-S, *et al*. Impact of virulence genes on sepsis severity and survival in *Escherichia coli* bacteremia. *Virulence*. 2015; **6**(1):93-100. doi:10.4161/21505594.2014.991234.
20. Ananias M, Yano T. Serogroups and virulence genotypes of Escherichia coli isolated from patients with sepsis. *Brazilian J Med Biol Res* 2008; **41**: 877-83; PMID:19030710; http://dx.doi.org/10.1590/S0100-879X2008001000008
21. Johnson JR, Stell AL. Extended virulence genotypes of Escherichia coli strains from patients with urosepsis in relation to phylogeny and host compromise. *J Infect Dis* 2000; **181**:261-72; PMID:10608775; http://dx.doi.org/10.1086/315217
22. Laupland KB, Gregson DB, Church DL, Ross T, Pitout JDD. Incidence, risk factors and outcomes of Escherichia coli bloodstream infections in a large Canadian region. *Clin Microbiol Infect* 2008; **14**:1041-7; PMID:19040476; http://dx.doi.org/10.1111/j.1469-0691.2008.02089.x [
23. Wiles T, Kulesus R, Mulvey MA. Origins and virulence mechanisms of uropathogenic Escherichia coli. *ExpMol Pathol,* 2008; **85**: 11-9; PMID:18482721; http://dx.doi.org/10.1016/j.yexmp.2008.03.
24. Rodríguez-Baño J, Mingorance J, Fernández-Romero N, Serrano L, López-Cerero L, Pascual A; ESBL-REIPI Group. Outcome of bacteraemia due to extended-spectrum â-lactamase-producing Escherichia coli: impact of microbiological determinants. *J Infect* 2013; **67**: 27-34; PMID:23588104; http://dx.doi.org/10.1016/j.jinf.2013.04.006
25. Mainil J. *Escherichia coli* virulence factors. *Veterinary Immunology and Immunopathology* 2013; **152**:2-12.
26. Bien J, Sokolova O, Bozko P. Role of Uropathogenic Escherichia coli Virulence Factors in Development of Urinary Tract Infection and Kidney Damage. *International Journal of Nephrology* 2012; 681473.
27. Tarchouna M, Ferjani A, Ben-Selma W, Boukadida J. Distribution of uropathogenic virulence genes in *Escherichia coli* isolated from patients with urinary tract infection. *International Journal of Infectious Diseases* 2013; **17**: e450-3.
28. Wang MC, Tseng CC, Chen CY, Wu JJ, Huang JJ.The role of bacterial virulence and host factors in patients with *Escherichia coli* bacteremia who have acute cholangitis or upper urinary tract infection. *Clinical Infectious Diseases* 2002; **15**: 1161-6.
29. Soto SM, Smithson A, Martinez JA, Horcajada JP, Mensa J, Vila J. Biofilm formation in uropathogenic Escherichia coli strains: relationship with prostatitis, urovirulence factors and antimicrobial resistance. *J Urol,* 2007; **177**: 365–8.
30. Pitout JDD, Laupland KB, Church DL, Menard ML, Johnson JR. Virulence Factors of *Escherichia coli* Isolates That Produce CTX-M-Type Extended-Spectrum â-Lactamases. *Antimicrob. Agents Chemother.* 2005; **49**: 4667-70.
31. Sharma S, Bhat GK, Shenoy S. Virulence factors and drug resistance in *Escherichia coli* isolated

from extraintestinal infections. *Indian Journal of Medical Microbiology,* 2007; **25**: 369-73.

32. Candan E D ,Aksöz N. *Escherichia Coli*: Characteristics of Carbapenem Resistance and Virulence Factors. *Braz. arch. biol. technol,* **60** Curitiba 2017; http://dx.doi.org/10.1590/1678-4324-2017160416
33. Johnson JR, Stell AL. Extended virulence genotypes of Escherichia coli strains from patients with urosepsis in relation to phylogeny and host compromise. *J Infect Dis* 2000; **181**: 261–72.
34. Nicolas-Chanoine MH, Blanco J, Leflon-Guibout V, Demarty R, Alonso MPM, Caniça M, *et al*. Intercontinental emergence of Escherichia coli clone O25: H4-ST131 producing CTX-M-15. *J Antimicrob Chemother,* 2007; **61**: 273–81.
35. Yang HH, Vinopal RT, Grasso D, Smets BF. High diversity among environmental Escherichia coli isolates from a bovine feedlot. *Appl Environ Microbiol* 2004; **70**:1528–36.
36. Harrison JJ, Ceri H, Yerly J, Stremick CA, Hu Y, Martinuzzi R, *et al*. The use ofmicroscopy and three-dimensional visualization to evaluate the structure of microbial biofilms cultivated in the Calgary Biofilm Device. *Biol Proced Online* 2006; **8**:194–215.
37. Olorunmola FO, Kolawole DO, Lamikanra A. Antibiotic Resistance and Virulence Properties in *Escherichia Coli* Strains from Cases of Urinary Tract Infections. *African Journal of Infectious Diseases*. 2013; **7**(1):1-7.