Research Article

Prevalence of Multi-drug resistant *Escherichia coli* isolated in neonatal intensive care units in a local hospital, Minia, Egypt

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Abstract
Neonatal sepsis is a leading cause of neonatal mortality in developing countries. Identification of the etiological agents of neonatal sepsis is essential for effective treatment. Out of 462 cases, 140 neonates had signs of sepsis, a total of 114/140 (81.4%) samples were positive for microbial growth. Gram positive cocci and Gram negative rods were isolated at the same rate (57/114, 50 % each) but *E. coli* was the most common (35.9%), followed by *S. aureus* (23.6%), coagulase negative Staphylococci (CONS) (20.11%). In addition, *E. coli* isolates showed highest resistance to cefotaxime (100%), linezolid (100%), ampicillin (97.5%), amoxicillin-clavulanic acid (97.5%), aztreonam (97.5%), cefepime (97.5%), meropenem (97.5), ampicillin-sulbactam,(82.9%), amikacin (75.6%), gentamicin (73.1%), piperacillin-tazobactam (73.1%) and ciprofloxacin (60.9%), least resistance was found to imipenem (34.1%) and azithromycin (34.1%). Also, of 41 E. coli isolates harbored 20 *hly* A genes, giving a 165-bp band and 19 *fim* H genes, giving a 508-bp band as shown by PCR.
Keywords: Neonatal sepsis, neonatal mortality, etiological agents, *Escherichia coli*

Introduction
Neonatal infections, particularly among very low birth weight, remain one of the major health problems and contribute to great mortality and morbidity (Thaver and Zaidi, 2009). Among these, bloodstream infections are a major contributor, being associated with the highest mortality (Simonsen et al., 2014).

Escherichia coli (*E. coli*) is an important pathogen associated with neonatal EOS and LOS, especially among VLBW infants (Simonsen et al., 2014; Weston et al., 2008). Neonatal pathogenicity due to extraintestinal pathogenic E. coli (ExPEC) is due to their possession of various virulence factors (VFs), which encoded on their chromosome, including adhesins or fimbriae (Type 1 Fimbriae, eg: fim A), iron-acquisition systems (also called siderophores), toxins (hemolysin, eg: hly A)., dispersin proteins and formation-related proteins, these virulence factors enhance their ability to cause systemic infections (Dobrindt, 2005; Jaureguy et al., 2008).

Methods
Study area and patients
This study was performed at the Neonatal Intensive Care Unit (NICU) at Minia General Hospital in the period from August 2016 until April 2017. Out of 462 cases, 140 neonates had signs of sepsis

Isolation and detection of causative neonatal sepsis
Blood samples were collected from newborns associated with one or more of signs of sepsis such as: lethargy, refusal of feeds, abdominal distension, respiratory distress, instability in temperature, pathological jaundice, convulsions and autonomic disturbances with constitutional symptoms . Two ml of venous blood collected from a peripheral vein of patients after adequate skin preparation and before the commencement of antibiotics. The blood was aseptically introduced into aerobic nutrient broth media and incubated for 2 to 7 days at 37^{o}C. After incubation, blood culture media were considered negative if there was no growth after

continuous incubation for up to 7 days, subcultures being made each day. Loopful from each positive blood culture was streaked on sterile plates of NA & SDA and incubated for 24 h at 37±2oC and 28±2oC for isolation of bacteria and fungi, respectively. After growth, obtained isolates of bacteria and fungi were identified according to Barrow & Feltham (2003).

Antibiotic susceptibility testing:

Antibiotic susceptibility tests were performed using Kerby-Bauer disc diffusion method on Mueller-Hinton agar according to the standards of Clinical and Laboratory Standards Institute (CLSI). The antibiotic discs including: Ampicillin AMP (10µg) Ampicillin – Sulbactam SAM (10⁄10 µg) Amoxicillin – Clavulanic acid AMC (20⁄10 µg) Cefotaxime CTX (30µg) Cefepime CPM (30µg) Imipenem IPM (10µg) Meropenem MEM (10µg) Amikacin AK (30µg) Gentamicin CN (10µg) Azithromycin AZM (15µg) Ciprofloxacin CIP (5µg) Piperacillin-tazobactam TPZ (100⁄10 µg) and Aztreonam ATM (30µg).

Detection of virulence genes

The primer use was as described in table (1).

(hly A and fim H)

PCR amplification involved a 25-µL reaction mixture containing template DNA (2 µL boiled lysate, 4 mM $MgCl_2$, 0.8 mM each of 4 dNTPs, 0.6 µM each primer [concentration 0.3 µM], and 2.5 units AmpliTaq Gold in 1 × PCR buffer [Perkin Elmer, Branchburg, NJ]).

Reactions were heated to 95°C in an automated thermal cycler (PTC-100-96; MJ Research, Watertown, MA) for 12 min to activate the AmpliTaq Gold. This was followed by 25 cycles of denaturation (94°C, 30s), annealing (63°C, 30 s), and extension (68°C,3 min) and a final extension (72°C, 10min). Samples were electrophoresed in 2% agarose gels, then stained with ethidium bromide, destained with distilled water, and photographed by use of an ultraviolet transilluminator and digital capture system (Gel Doc; BioRad, Hercules, CA). The sizes of the amplicons were determined by comparing them with a 100-bp DNA ladder (Gibco/BRL, Gaithersburg, MD), which was run in multiple lanes on the same gel (Johnson and Stell, 2000).

Gene	Primers	Sequence	Product size (bp)	Source of reference
fimH	FimH- F FimH -R	5'-TGCAGAACGGATAAGCCGTGG-'3 5'-GCAGTCACCTGCCCTCCGGTA -3'	508-bp	**(Johnson and Stell, 2000)**
hlyA	hlyA- F hlyA- R	5'-ACGATGTGGTTTATTCTGGA-'3 5'-CTTCACGTGACCATACATAT-3'	165-bp	**(Kargar and Homayoon, 2015)**

Results

A total of 114/140 (81.4%) samples were positive for microbial growth. Gram positive cocci and Gram negative rods were isolated at the same rate (57/114, 50 % each) but *E. coli* was the most common (35.9%), followed by *S. aureus* (23.6%), coagulase negative Staphylococci (CONS) (20.11%).

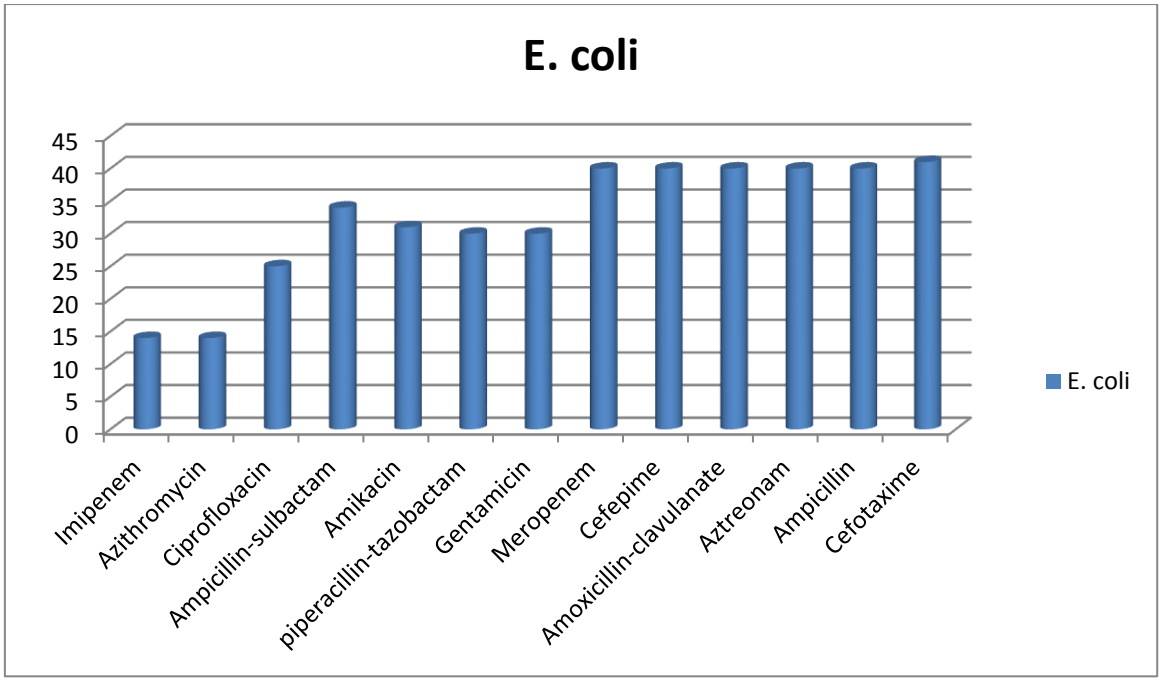

Figure 1 Resistance of *E. coli* to various antimicrobials

E. coli isolates showed highest resistance to cefotaxime (100%), linezolid (100%), ampicillin (97.5%), amoxicillin-clavulanic acid (97.5%), aztreonam (97.5%), cefepime (97.5%), meropenem (97.5), ampicillin-sulbactam,(82.9%), amikacin (75.6%), gentamicin (73.1%), piperacillin-tazobactam (73.1%) and ciprofloxacin (60.9%), least resistance was found to imipenem (34.1%) and azithromycin (34.1%) as shown in figure 1.

As shown by PCR, of 41 E. coli isolates harbored 20 *hly* A genes, giving a 165-bp band and 19 *fim* H genes, giving a 508-bp band.

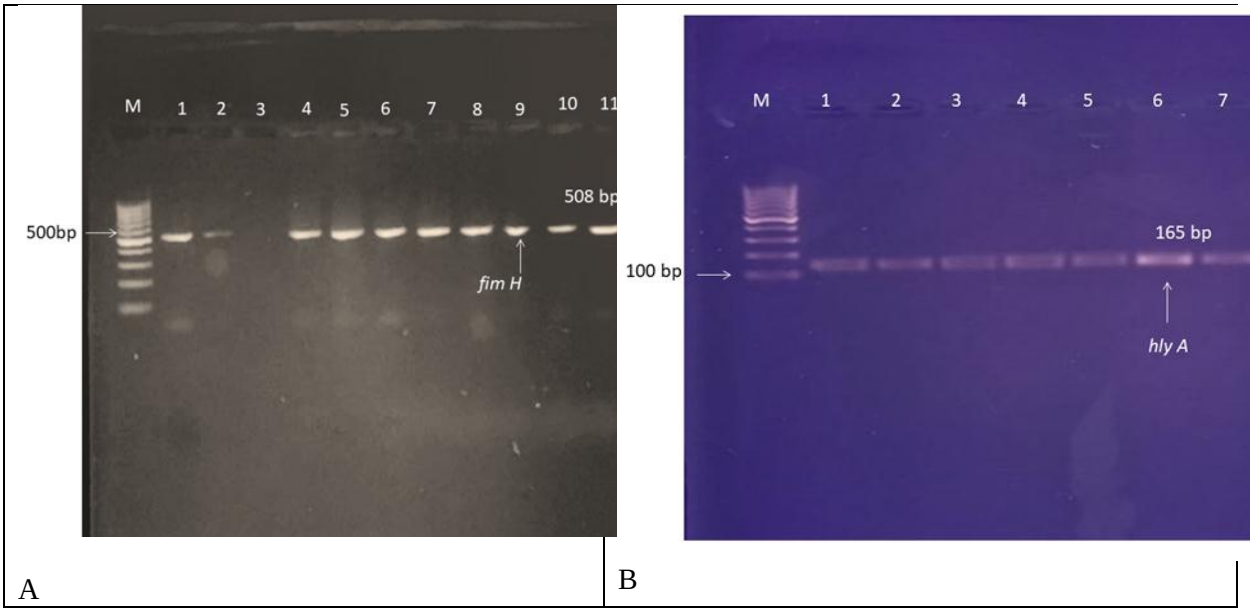

Figure 2: PCR detection of fim H (A) and hly A (B) genes. Lane M, molecular size marker (100 bp Ladder); lanes 1,2,4,5,6,7,8,9,10 and 11, *fim* H at 508 bp; lane 3 negative and lanes 1,2,3,4,5,6 and 7, *hly* A at 165bp.

Discussion

Among neonates, *E. coli* has been associated with approximately 18% of early-onset sepsis and a similar percentage of late-onset sepsis, placing it as the second or third most common etiology in the NICU (Vergnano et al., 2011), EOS due to E. coli usually has a higher mortality rate than that caused by gram-positive bacteria (Simonsen et al., 2014). In the present study 41(35.9%) E. coli were isolated from blood cultures causing neonatal sepsis. (Al-Matary et al., 2019) found that amongst neonates with EOS, E. Coli (27.3%) was the second most frequently detected pathogen after CoNs, this finding is partially agree with our results. While other study in a perinatal center in eastern China which Conducted on two periods, 2002 to 2008 and 2012 to 2018 reported that E. coli continued to be the leading bacterial pathogen for EOS, (32.3%) in 2002–2008 and (26.5%) in 2012–2018 and this close to our results (Zhu., 2019).

In another study performed by (Abdel-Wahab et al., 2013) in an Egyptian neonatal intensive care unit had shown that Escherichia coli accounted for (21.6%) of isolates causing neonatal blood stream infection, this is in partial agreement with our results.

Previously, *E. coli* was widely susceptible to a range of antibiotics, but, in the past decade, resistance has increased in prevalence, in a study performed in Taiwan, (79%) from *E coli* isolates were ampicillin-resistant, (16%) were gentamicin-resistant and no cases of third-generation cephalosporin-resistant *E coli* (Tsai et al., 2012). On the other hand, the antimicrobial resistance pattern in Delhi NICU, India appears to be even worse, high rates of multidrug resistance were observed in *E. coli* (38%) isolates (Agarwal and Sankar, 2016).

In published studies in China, the antibiotic resistant patterns to several antibiotics were observed (Li et al., 2018), from these data, (79.5%) of *E. coli* responsible for neonatal septicemia were ampicillin-resistant,cefotaxime (45%), aztreonam (51.5%), ampicillin-sulbactam, (55.2%), gentamicin (49.1%), ciprofloxacin (35.4%), amikacin (18%), meropenem (14.7), and least resistance was found to imipenem (8.8%). Obtained results were relatively in agreement with those found by (Aamir et al., 2015), in which, more than 60% of E. coli strains are resistant to ampicillin, amoxicillin-clavulanic acid, ampicillin-sulbactam, cefepime, cefotaxime, ciprofloxacin, gentamicin and imipenem antibiotics. In contrast, less than 50% of E. coli strains are resistant to amikacin and piperacillin - tazobactam antibiotics.

The presence of *hly* A and *fim* H genes in E. coli strains isolated from neonatal blood samples, giving a 165-bp for *hly* A and a 508-bp for *fim* H genes indicate the role of *hly* A and *fim* H genes as a virulence factors that promote extraintestinal infection.

Chmielarczyk et al., (2013) reported that the most frequently detected adhesion genes were *fim* H (75%) from the isolated *E. coli* isolated from LOI, mainly from pneumonia and BSI from Polish NICUs. This result close to that obtained in this study. The findings of this study also are consistent with those of another study, which showed a high incidence of *hly* A and *fim* H genes, were found to be associated with strains isolated from blood culture and thus may be of importance in septicemia (Watt et al., 2003). Nojoomi and Ghasemian (2019) found that most of the *E. coli* strains that caused septicaemia in paediatric patients in five hospitals in Tehran, there was a higher prevalence of *hlyA* genes.

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