

Original Research Article

Prevalence of Multidrug Resistant Bacteria Causing Late-Onset Neonatal Sepsis

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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 106 microbial isolates recovered from blood cultures of late onset neonatal sepsis (LONS) patients, only 70 (66 %) isolates were Gram-positive bacteria, 31 (29.2%) isolates were Gram-negative bacteria and 5 (4.7%) isolates were *Candida* sp. Coagulase negative staphylococci (CONS) was the most common causative LONS, which reached to 43(40.6%) of total isolates, followed by *Micrococcus*, *Enterobacter*, Coagulase positive staphylococci (COPS), *Candida*, *Shigella*, *E. coli*, *Bacillus*, *Citrobacter* and *Klebsiella*, which reached to 13(12.3%), 11(10.4%), 10(9.4%), 7(6.6%), 5(4.7%), 5(4.7%), 4(3.8%),4(3.8%) and 4(3.8%), respectively. CONS strains appeared more resistance to various test antibiotics compared to COPS. In addition, both of *Staphylococcus* and *Enterobacterial* isolates appeared high degree of resistance to penicillins and cephalosporins antibiotics, but their sensitivity to other tested antibiotics are different. In addition, about 50% of *Staphylococcus* isolates were resistant to aminoglycosides, IPM, glycopeptides and linezolid antibiotics, while 50% *Enterobacterial* isolates were resistant to glycopeptides, aminoglycosides, monobactam and tetracycline. The most resistant strains in the present study are *Staphylococcus* NBS-35, *Staphylococcus* NBS-98 and *Enterobacter* NBS-40 which identified based on 16S rRNA gene sequence as strains of *Staphylococcus epidermidis*, *Staphylococcus aureus subsp. aureus* and *Enterobacter cloacae*, respectively.

Keywords

Neonatal sepsis,
resistant,
antibiotics,
Staphylococcus,
Enterobacter

Introduction

Neonatal sepsis refers to systemic infection of the newborn. It is characterized by a constellation of a nonspecific symptomatology in association with bacteremia. Prompt recognition, appropriate antimicrobial therapy and judicious supportive care are the key determinants of positive outcome in this serious pediatric emergency. In developing countries, sepsis including meningitis, respiratory infections, diarrhea, and neonatal tetanus is the commonest cause of mortality responsible for 30-50 % of 5 million total neonatal deaths each year. It is estimated that almost 20 % of all neonates develop infection and approximately 1 % die of the serious systemic infections. Not surprisingly, sepsis is the commonest admitting diagnosis among neonates at referral facilities (Paul and Singh, 2000 and Remington *et al.*, 2006).

The detection of microorganisms in a patient's blood has a great diagnostic and prognostic significance. Blood cultures provide essential information for the evaluation of a variety of diseases like endocarditis, pneumonia, and pyrexia of unknown origin and particularly, in patients with suspected sepsis (Murty and Gyaneshwari, 2007). Pathogens causing neonatal infections and their antibiotic susceptibility patterns may change over time and differ between countries (Anwer *et al.*, 2000; May *et al.*, 2005; Zaidi *et al.*, 2005). It is extremely important to diagnose the cases in time so that appropriate antibiotic treatment can be given. Moreover, neonatal infection surveillance networks have been established in several countries and are useful for documenting changes in clinical practice, monitoring changes in pathogens and their antibiotic resistance over time, informing policy and improving quality of

care. Thus, the bacterial pathogens responsible and their susceptibility pattern should be regularly monitored in a hospital setting (Mahmood *et al.*, 2002 and Gray, 2007).

Recently, there have been an increase in antibiotic resistance over the past two decades which is due to mutant forms of common bacteria, overuse, or under use or inappropriate use of broad spectrum antibiotics and poor infection control in maternity and neonatal units (Dzidic *et al.*, 2003; Waheed *et al.*, 2003; Tom-Revzon, 2004; Aftab and Iqbal, 2006 and Muhammad *et al.*, 2010). The present study aim to describe the distribution of different causative late onset neonatal sepsis and identification of the most resistant isolates of bacteria using 16S rRNA gene sequence.

Materials and Methods

Blood samples

The prospective study was conducted on culture blood samples collected from neonatal intensive care unit at Almaza Hospital, Cairo, Egypt, during 12 months period from December 2011 to December 2012.

Media, Antibiotic disks and PCR kits

Nutrient agar (NA), Sabouraud dextrose agar (SDA), Mueller Hinton Agar (MHA), Penicillin (P) 10 units, Ampicillin (AMP) 10µg, Amoxicillin/Clavulanic acid (AMC) 20/10µg, Cefotaxime (CTX) 30µg, Ampicillin/Sulbactam (SAM) 10/10µg, Oxacillin (OX) 1µg, Cefazidime (CA) 30µg, Cefepime (CPM) 30µg, Naldixic acid (NA) 30 µg, Clindamycin (DA) 2µg, Ofloxacin (OFX) 5µg, Clarithromycin (CLR) 15µg, Vancomycin (VA) 30µg, Piperacillin/Tozobactam

(TPZ)100/10µg Ciprofloxacin (CIP)5µg, Tetracycline (T) 30µg, Doxycycline (DO) 30µg, Gentamycin (CN)10µg, Linezolid (LZD)30µg, Norfloxacin(NOR)10µg, Streptomycin(S) 10µg, Imipenem(IPM) 10µg, Amikacin (AK)30µg and Tobramycin (TOB)10µg/disk were purchased from Oxoid Ltd Co. GeneJet genomic DNA purification Kit (Thermo K0721), 1Kb plus ladder (Thermo) GeneJET™ PCR Purification Kit (Thermo K0701) and universal primers (8F: AGA GTT TGA TCC TGG CTC AG & U1492R: GGT TAC CTT GTT ACG ACT T) were purchased from Sigma Scientific Services Co. Egypt

Isolation and detection of causative neonatal sepsis

Blood samples were collected from newborns (more than 72h) 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 °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±2°C and 28±2°C 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 testing was applied only on bacterial isolates listed in Clinical and Laboratory Standards Institute (CLSI) (2011), while other microorganisms such as *Bacillus* or *Micrococcus* would not be studied. Antibiotic disks including P, AMP, AMC, CTX, SAM, OX, CA, CPM, NA, DA, OFX, CLR, VA, TPZ, CIP, T, DO, CN, LZD, NOR, S, IPM, AK and TOB were applied on Gram positive isolates, while AMP, AMC, CTX, SAM, CA, CPM, NA, OFX, TPZ, CIP, T, DO, CN, NOR, S, IPM, AK and TOB were applied on Gram negative isolates according to CLSI (2011).

Identification of the most resistant isolates

The most resistant strains of causative LONS were identified as follow: DNA was extracted from tested bacterial strains using GeneJet genomic DNA purification Kit (Thermo K0721) according to manufacturer's instructions. After extraction, 5µl of extracted DNA was used as a template for each 50 µl PCR reaction. PCR reaction was performed with 25µl of Maxima® Hot Start PCR Master Mix (2X), 1µl (20µM) of each primer 8F: AGA GTT TGA TCC TGG CTC AG & U1492R: GGT TAC CTT GTT ACG ACT T; 5µl of template DNA and 18 µl of water, nuclease-free. The reaction was performed in a thermocycler (Biometra) as follows: one cycle of 10min at 95°C; 35 cycles of 30s at 95°C, 60s at 65°C & 90s at 72°C and one cycle of 10min. PCR products were observed by loading of 4µl of PCR product against 1Kb plus ladder (Thermo) on agarose (1.0%) gel electrophoresis PCR. PCR product were clean up GeneJET™ PCR Purification Kit (Thermo K0701) according to manufacturer's instructions.

The Basic Local Alignment Search Tool (BLAST) data base (Altschul *et al.*, 1997) of National Center for Biotechnology (NCBI) Information was used to compare the sequence of 16S rDNA of the most resistant strains with known 16S rDNA sequences of bacteria, then, obtained alignments were constructed using phylogeny.fr program (Dereeper *et al.*, 2008).

Result and Discussion

The present study was applied on blood samples of neonatal sepsis patients of Almaza Hospital during the period from December 2011 to December 2012.

Data recoded in Table (1) revealed that out of 106 microbial isolates recovered from blood cultures of LONS patients, only 70(66 %) isolates were Gram-positive bacteria, 31 (29.2%) isolates were Gram-negative bacteria and 5(4.7%) isolates were *Candida* sp. In addition, out of 67 microbial isolates recovered from blood cultures of male patients, only 43(64.2%) Gram-positive bacteria, 21 (31.3%) isolates were Gram-negative bacteria and 3 (4.5%) isolates were *Candida* sp. Moreover, out of 39 microbial isolates recovered from blood cultures of female patients, only 27(69.2%) Gram-positive bacteria, 10 (25.6%) isolates were Gram-negative bacteria and 2 (5.1%) isolates were *Candida* sp.

In the case of male blood samples, CONS was the most common causative LONS, which attained to 24(35.8%) followed by *Micrococcus*, *Enterobacter*, COPS, *Shigella*, *E.coli*, *Bacillus*, *Candida*, *Citrobacter* and *Klebsiella*, which attained to 9 (13.4%), 7(10.4%), 6(9.0%), 6(9.0%),5(7.5%), 4(6.0%), 3(4.5%), 2(3.0%) and 1(1.5%), respectively. While, In case of female blood

samples, CONS was the most common causative LONS, which attained to 19(48.7%), followed by COPS, *Micrococcus*, *Enterobacter*, *Klebsiella*, *Candida*, *Citrobacter* and *Shigella*, which reached to 4(10.3%), 4(10.3%), 4(10.3%), 3(7.7%), 2(5.1%), 2(5.1%) and 1(2.6%), respectively (Table,1).

From all abovementioned results, it could be concluded that CONS was the most common causative LONS, which reached to 43(40.6%) of total isolates, followed by *Micrococcus*, *Enterobacter*, COPS, *Shigella*, *Candida*, *E.coli*, *Bacillus*, *Citrobacter* and *Klebsiella*, which reached to 13(12.3%), 11(10.4%), 10(9.4%), 7(6.6%), 5(4.7%), 5(4.7%), 4(3.8%),4(3.8%) and 4(3.8%), respectively, (Table,1). Furthermore, the most common member of family enterobacteriaceae causing LONS is *Enterobacter* followed by *E. coli*, *Citrobacter* and *Klebsiella*.

Antibiotic susceptibility testing

Data recorded in Table (2) reveal that most of causative LONS isolates are resistant to various tested antibiotics. In case of CONS, more than 50% of isolates are resistant to P, AMP, AMC, SAM, OX, CPM, CTX, CA, NA, DA,CIP, CLR, OFX and VA antibiotics. In contrast, less than 50% of CONS strains are resistant to IPM, NOR, T, CN, TPZ, AK, LZD, TOB, S and DO antibiotics. In case of COPS strains more than 50% of isolates are resistant to P, AMP, AMC, SAM, OX, CPM, CTX, CA, DA, OFX and VA antibiotics. In contrast, less than 50% of COPS strains are resistant to IPM, NOR, T, CN, TPZ, AK, LZD, TOB, S, NA, CIP, CLR, and DO antibiotics (Table, 2).

Data presented in Table (2) revealed that more than 50% of *Enterobacter* strains are resistant to AMP, AMC, SAM, CPM, CTX,

CA, NA, CIP, NOR, CN, TPZ, TOB, S and OFX antibiotics. In contrast, less than 50% of *Enterobacter* isolates are resistant to IPM, T, AK and DO. In case of *Shigella* strains, more than 50% of them are AMP, AMC, SAM, CPM, CTX, CA and IPM antibiotics. In contrast, less than 50% of *Shigella* strains are resistant to NA, CIP, OFX, NOR, T, CN, TPZ, AK, TOB, S and DO antibiotics. In case of *E. coli* strains, more than 60% of strains are resistant to AMP, AMC, SAM, CPM, CTX, CA, IPM, NA, CIP, OFX, NOR, T, CN, TOB, and S antibiotics. In contrast, less than 50% of *E. coli* strains are resistant to TPZ, AK and DO antibiotics. In case of *Citrobacter* strains, more than 50% of them are AMP, AMC, SAM, CPM, CTX, CA, OFX, T, CN, AK, DO and S antibiotics. In contrast, less than 50% of *Citrobacter* strains are resistant to IPM, NA, CIP, NOR, TPZ and TOB antibiotics. In case of *Klebsiella* strains, more than 50% of strains are resistant to AMP, AMC, SAM, CPM, CTX, CA, OFX, T, CN, AK, DO, IPM, NA, CIP, NOR and TOB antibiotics. In contrast, less than 50% of *Klebsiella* strains are resistant to TPZ and S antibiotics.

From previous results, it could be summarized that CONS strains appeared more resistance to various test antibiotics compared to COPS. In addition, *Enterobacter* and *Shigella* isolates appeared resistance to various tested antibiotics compared to other gram negative bacteria which their resistance were ranged from 0 to 100%.

Overall antibiotics susceptibility pattern of *Staphylococcus* isolates

Data illustrated in Fig.(1) showed that the maximum percentage of resistant *Staphylococcus* isolates to antibiotics was detected with P (94.3%) followed by AMP (88.7%), AMC (77.4%), CTX (77.4%),

SAM (77.4%), OX (75.5%), CA (73.6%), CPM (73.6%), NA (62.3%), DA (58.5%), OFX (56.6%), CLR (54.7%), VA (54.7%), TPZ (50.9%), CIP (45.3%), T (45.3%), DO (41.5%), CN (34.0%), LZD (34.0%), NOR (34.0%), S (34.0%), IPM (28.3%), AK (22.6%), and TOB (22.6%).

In addition, the maximum percentage of Gram positive bacterial isolates intermediate to antibiotics was detected with CA (13.2%) followed by CIP (13.2%), VA (11.3%), CTX (9.4%), DO (7.5%), LZD (7.5%), NOR (7.5%), S (7.5%), TPZ (7.5%), IPM (5.7%), CLR (3.8%), CPM (3.8%), DA (1.9%), AK (1.9%), CN (1.9%), NA (1.9%), OX (1.9%), TE (1.9%) and TOB (1.9%) (Fig.,1). On the other hand the maximum percentage of sensitive Gram positive bacterial isolates to antibiotics was detected with AK (75.5%) and TOB (75.5%), followed by IPM (66.0%), CN (64.2%), S (58.5%), NOR (58.5%), LZD (58.5%), T (54.7%), DO (50.9%), OFX (43.4%), TE (43.4%), CIP (41.5%), CLR (41.5%), TPZ (41.5%), DA (39.6%), NA (35.8%), VA (34.0%), CPM (22.6%), OX (22.6%), SAM (22.6%), AMC (22.6%), CTX (13.2%), CA (13.2%), AMP (11.3%) and P (5.7%)(Fig.,1).

Overall antibiotics susceptibility pattern of *enterobacterial* isolates.

Data illustrated in Fig.(2) show that the maximum percentage of resistant Gram negative bacterial isolates to antibiotics was observed with AMP (96.8%) followed by AMC (80.6%), SAM (80.6%), CA (71%), CPM (71.0%), CTX (71.0%), TOB (64.5%), NA (61.3%), CN (55.0%), CIP (58.1%), OFX (58.1%), T (48.4%), IPM(45.2%), S (42%), DO (41.9%), AK (38.7%), NOR (32.3%) and TPZ(29.0%).

In addition, the maximum percentage of intermediate Gram negative bacterial isolates to antibiotics was detected with

CPM (12.9%), CIP (12.9%), CTX (9.7%), NA (9.7%), TPZ (9.7%), T (9.6%), NOR (3.2%), S (3.2%) and CN (3.0%) (Fig.,2). On the other hand the maximum percentage of sensitive Gram negative bacterial isolates to antibiotics was detected with NOR (64.5%) followed by AK (61.3%), TPZ (61.3%), DO (58.1%), S (54.8%), IPM (54.8%), CN (42.0%), T (42.0%), OFX (41.9%), TOB (35.5%), CA (29%), CIP (29%), NA (29%), AMC (19.4%), SAM (19.4%), CTX (19.3%), CPM (16.1%) and AMP (3.2%)(Fig.,2).

From all abovementioned results, it could be concluded that all tested strains were resistant to various tested antibiotics but with different level of resistance based on the type and group of antibiotic used. Furthermore, tested staphylococcal isolates displayed a high degree of resistance to most penicillins and cephalosporins antibiotics but aminoglycosides, IPM, glycopeptides and linezolid were relatively effective to more than 50% of isolates. While, tested enterobacterial isolates displayed a high degree of resistance to most penicillins and cephalosporins antibiotics but glycopeptides, aminoglycosides, monobactam and tetracycline were relatively effective to more than 50% of isolates.

Identification of the most resistant isolates

Data illustrated in Fig(3) revealed that *Staphylococcus* NBS-35 and *Staphylococcus* NBS-98 were appeared the maximum percentage of resistant to various tested antibiotics, which reached their resistance to 100 and 88% of all tested antibiotics, respectively.

In addition, Data illustrated in Fig.(4) reveal that the phylogenetic of the partial 16S ribosomal RNA genes sequence of

Staphylococcus-NBS-035 is matched with the reference sequence of *Staphylococcus aureus subsp. aureus* N315 in BLST data base, thus, *Staphylococcus*-NBS-35 is identified as a strain of *Staphylococcus aureus subsp. aureus* (Fig.4). Furthermore, the phylogenetic of the partial 16S ribosomal RNA genes sequence of *Staphylococcus*-NBS-98 is matched with the reference sequence of *Staphylococcus epidermidis* ATCC 12228 in BLST data base thus, *Staphylococcus*-NBS-98 is identified as a strain of *Staphylococcus epidermidis* (Fig.5).

Data illustrated in Fig (6) reveal that *Enterobacter* NBS-40 showed the maximum percentage of resistant to various tested antibiotics, which reached their resistance to 58.1 of all tested antibiotics.

Data illustrated in Fig.(7) revealed that the phylogenetic of the partial 16S ribosomal RNA genes sequence of *Enterobacter*-NBS-40 is matched with the reference sequence of *Enterobacter cloacae* SCF1 in BLST data base, thus, *Enterobacter*-NBS-40 is identified as a strain of *Enterobacter cloacae*.

Neonatal septicemia remains one of the most causes of mortality and morbidity despite considerable progress in hygiene, introduction of new and potent antimicrobial agents and advanced measures for diagnosis and treatment (Bizzarro *et al.*, 2005). Up to 10% of infants have infections in the first month of life which are responsible for 30 - 50% of total neonatal deaths in developing countries (Donowitz, 1989 and Stoll *et al.*, 2002).

In the present study, the etiological agents of LONS was diverse, which includes Gram positive bacteria, Gram negative bacteria and *Candida*. In addition, CONS showed the highest Gram positive organisms, followed

by *Micrococcus*, *COPS* and *Bacillus*. Furthermore, *Enterobacter* was the most common member of family *enterobacteriaceae* recovered from tested blood cultures followed by *E. coli*, *Citrobacter* and *Klebsiella*. LONS is caused by microorganisms thriving in the external environments of homes or hospitals (Amdekar, 2006) and that may be explained the wide variety of microorganisms recovered from patients infected by LONS in the present study. In addition, high distribution of coagulase negative staphylococci as a causative agent of LONS may be due to some reasons such as CONS are common inhabitants of the skin and mucous membranes; although a small proportion of neonates acquire CoNS by vertical transmission, acquisition primarily occurs horizontally (Hallet *et al.*, 1990 and Patrick *et al.*, 1992). Consequently, infants admitted to a hospital obtain most of their microorganisms from the hospital environment, their parents, and staff (Huebner *et al.*, 1999 and Remington *et al.*, 2006). The obtained results are in agreement with those observed by other studies with different level of percentage. For instance, Wu *et al.* (2009) reported that Gram positive organisms account for about 70% of all late onset sepsis and the most common causative microorganisms in LONS were CONS (40%). Stoll *et al.* (2002) detected the same previous results but with 48% of infections by CONS. While, Downey *et al.* (2010) reported that the majority (45%-75%) of pathogens responsible for LOS are Gram-positive bacteria and the most common organism isolated in LONS, coagulase-CONS is also the overall least virulent. Groups from England, Israel, and the United States have all reported similar rates (47%-54%) of LONS infections secondary to CONS in the past decade (Bizzarro *et al.*, 2005).

Detection of *Micrococcus* and *Bacillus* as causative organisms in neonatal sepsis is rare in both developed and developing countries, but in our study *Micrococcus* and *Bacillus* were the second and fourth frequent causative organisms, respectively, that may be due to lack of applied standard infection control precautions, especially hand hygiene in intensive care unit, especially in developing country (Long, 2003 and Fahmey, 2013).

Current study showed that a very high degree of resistance of staphylococcal isolates to various generations of beta lactam and cephalosporin antibiotics. The highest antibiotic resistance was detected with P and AMP followed by AMC, SAM and OX, while IPM and TPZ appeared the lowest frequent antibiotics resistant among tested staphylococcal isolates. In addition, more than 50% of tested of staphylococcal isolates were VA and TE antibiotics. High prevalence of resistant staphylococcal isolates to various generations of beta lactam and cephalosporin antibiotics, especially OX may be due to the extensive use of third generation cephalosporins as first and second-line drugs against Gram positive bacteria in neonatal intensive care units around the world (Moolenaar *et al.*, 2000; Ebelechukwu, 2003 and West & Peterside, 2012). In addition, a high prevalence of methicillin-resistant *Staphylococcus* ssp. in NICUs usually leads to frequent vancomycin use. Concerns have been raised about the spread of vancomycin hetero-resistant *S. capitis* strains in NICUs and their involvement in persistent bacteremia despite prolonged vancomycin therapy (Van Der Zwet *et al.*, 2002; Ng *et al.*, 2006 and D'Mello *et al.*, 2008).

The obtained results were in agreement with many investigations in developing countries: in Egypt, Mahmood *et al.* (2002) reported

that 100% of *Staphylococcus* isolates were found resistant to ampicillin. In India, both *S. aureus* and *S. epidermidis* had shown almost complete resistance to penicillin (93.75%) (Sheth *et al.*, 2012). Resistance of *Staphylococcus* isolates to cefotaxime and ceftazidime were ranged from 34 to 86% and from 40 to 71.6%, respectively (Mokuolu *et al.*, 2002 and Aurangzeb and Hameed, 2003). In another studies, resistance of *Staphylococcus* isolates to ampicillin and amoxicillin were attained to 90% (Bhurle and Solabannavar, 2014), 83.3% (Waseem *et al.*, 2005) and 77% (Muhammad *et al.*, 2010). Muhammad *et al.*, (2010) found that Gram-positive and negative bacteria had demonstrated high resistance against third generation cephalosporins, such as cefotaxime (63.1%) and ceftriaxone (66.9), whereas ceftazidime was resistant in 56.9% of the neonatal sepsis cases. In addition, methicillin resistant *Staphylococcus* isolates in the present study was relatively high (75 %) compared to other studies, which found that 62.7% (Singhal *et al.*, 2006), 61.54% (Mahmood *et al.*, 2002), 44.4% (Elmashad *et al.*, 2009) and 41.2% of the *S. aureus* species and 50% of coagulase negative *Staphylococcus* species (Gebrehiwot *et al.*, 2012). While other studies reported that a high percentage of *S. epidermidis* and *S. haemolyticus* isolates coming from neonates were resistant to oxacillin, which reached to 92% and 100%, respectively (Villari *et al.*, 2000; Qu *et al.*, 2010 and Abd El Hafez *et al.*, 2011).

On the other hand, resistant of staphylococcal isolates to some beta lactam and cephalosporin antibiotics was relatively low compared to our results. For instance, Kaistha *et al.* (2009) found that the sensitivity of *Staphylococcus* isolates to cefotaxime and ceftriaxone were reached to 66 and 86.4%, respectively. In addition, Sheth *et al.* (2012) reported that the sensitivity of *S. aureus* and

S. epidermidis to cloxacillin was exceeded 50% as reported in India, Nepal and Saudi Arabia (Shaw *et al.*, 2007; Abd El Hafez, 2008 and Raghunath, 2008). Moreover, Singhal *et al.* (2006) found that 58.8% of *S. aureus* isolates were sensitive to penicillin. In the case of VAN: many investigations revealed that the sensitivity of Gram positive isolates to VAN attained to 100% (Mahmood *et al.*, 2002; Singhal *et al.*, 2006; Desai *et al.*, 2011 and Shah *et al.*, 2012). In addition, susceptibility of Gram positive isolates to VAN was reached to 95% (Shrestha *et al.*, 2013) and 84% (Bhurle and Solabannavar, 2014). Furthermore, Najeeb *et al.* (2012) had found 100% sensitivity of VAN against *Staph. aureus* and *Staph. Epidermidis*. In Egypt, among *Staph. aureus* isolated strains, MRSA was detected by oxacillin disc diffusion method in 44.4% in late onset sepsis cases (Elmashad *et al.*, 2009). While, in Tanzania, Kayange *et al.* (2010) detected that 28% of *S. aureus* were MRSA, while, Bhurle and Solabannavar (2014) found that 100% of staphylococcal isolates were detected as MRSA. In the case of imipenem, Shaw *et al.* (2007) and Waseem *et al.* (2005) have described 100% sensitivity of imipenem against *Staphylococcus*. Shrestha *et al.* (2013) found that 75% of Gram positive bacteria was susceptible to imipenem antibiotic.

High prevalence of bacterial resistance to aminoglycoside in the present study may be due to use of these antibiotics with beta lactam and cephalosporin antibiotics as first-line drugs in neonatal intensive care units around the world (Moolenaar *et al.*, 2000 and West and Tabansi, 2014). Obtained results are almost similar to the foundation by Singhal *et al.* (2006), who revealed that 22.8% of staphylococcal isolates were resistant to AK. In addition, Shrestha *et al.* (2013) noted that among Gram positive isolates 31.5% was resistance to CN. While,

other studies found that 80% of *S. epidermidis* and 100% of *S. haemolyticus* isolates were resistant to gentamicin (Villari *et al.*, 2000; Qu *et al.*, 2010 and Abd El Hafez *et al.*, 2011), Bhurle and Solabannavar (2014) reported that 70% of Gram positive isolates were resistant to CN and AK, 50 % of staphylococcal isolates were resistant to CN Kaistha *et al.* (2009). In most of the neonatal sepsis cases, causative organisms were found to be resistant to CN had reached to 55.1% cases, while AK and TOB had relatively less resistance (17.4 and 34.8% cases, respectively) (Najeeb *et al.*, 2012).

Although fluoroquinolone, T and DO were not frequently used for neonatal sepsis, but resistant was emerging against them because of indiscriminate use of antibiotics (Shaw *et al.*, 2007 and Najeeb *et al.*, 2012). In the case of fluoroquinolone, the obtained results were in agreement with some investigations, Najeeb *et al.* (2012) revealed that 40 % and 38.5% of *S. hemolyticus* isolates were resistant to ciprofloxacin and ofloxacin, respectively. In addition, Gebrehiwot *et al.* (2012) reported that 35.9% of *S. aureus* isolates were resistant. On the other hand, resistant of *S. hemolyticus* and CONS to ciprofloxacin attained to 72.7 and 22.6%, respectively (Singhal *et al.*, 2006). Furthermore, 13% of CoNS isolates was resistant to ofloxacin (Bhurle and Solabannavar, 2014).

Distribution of macrolide, lincosamide and linezolid resistant staphylococcal isolates in our study may be due to the recommended as first or second-line agents and alternative against staphylococcal infections, mainly in penicillin-allergic patients or in MRSA-infected patients (Gemmell *et al.*, 2006; Grayson, 2006). Obtained results are relatively low compared to previous studies, which revealed that among *S. epidermidis*

strains, 90% of strains were resistant to erythromycin & 39% to clindamycin, and among *S. haemolyticus* isolates, 100% were resistant to erythromycin and 18% to clindamycin (Brzychczy- Wloch *et al.*, 2013). In addition, erythromycin resistance attained to 30% (Sheth *et al.*, 2012) against 46.5% in another study (Kaistha *et al.*, 2009). On contrast, Bhurle and Solabannavar (2014) reported that the Gram positive bacteria showed high susceptible to Azithromycin (90%). Furthermore, Shah *et al.* (2012) and Brzychczy-Wloch *et al.* (2013) found that sensitivity of tested staphylococcal isolates attained to Linezolid was sensitive in all isolates (100%).

In the present study, 29.2% of causative neonatal sepsis due to family enterobacteriaceae, represented by *Enterobacter*, *Shigella*, *E. coli*, *Citrobacter* and *Klebsiella*. Hevas (2001) reported that coliform organisms are prevalent in the maternal birth canal, and most infants are colonized in the lower gastrointestinal or respiratory tracts during or just before delivery. Furthermore, Kangozhinova *et al.* (2013) reported that predominance of Gram-negative organisms due to the indiscriminate and inappropriate use of antibiotics, lack of hygienic practices at the place of delivery, poor cord care and unhygienic newborn care practices. Obtained results are comparable with those detected by other studies, which found that neonatal sepsis caused by Gram-negative microorganisms is responsible for 18%–78% of all neonatal sepsis during 10 years (Couto *et al.*, 2007; Macharashvili *et al.*, 2009 and Kamath *et al.*, 2010). Recent studies have indicated that the incidence of Gram-negative bacterial infections in neonatal intensive care units may be increasing (Nambiar *et al.*, 2002 and Kristof *et al.*, 2009). On the other hand, Kangozhinova *et al.* (2013) found that

57.5 % of late onset sepsis caused by members of family enterobacteriaceae.

Determination of antibiotic sensitivity patterns in periodic intervals is mandatory in each region for choosing appropriate antibiotic therapy (Karki *et al.*, 2010 and Rathod *et al.*, 2012). According to food and drug administration (FDA), approximately 21 to \$ 34 billion are attributable to treat infections due to antimicrobial resistance pathogens in USA (Spellberg *et al.*, 2011). Current study showed that a very high degree of resistant of enterobacterial isolates to various generations of beta lactam and cephalosporin antibiotics, while, resistance to TPZ and IPM appeared the lowest frequent among tested enterobacterial isolates. In addition, resistance of enterobacterial isolates to various tested aminoglycoside, tetracycline and fluoroquinolone antibiotics is relatively high. The high percentage of resistant causative neonatal sepsis in the present study may due to some reasons including: the extensive use of third generation cephalosporins as first and second-line drugs

against gram negative bacteria in neonatal intensive care units around the world (Ebelechukwu, 2003; West and Peterside, 2012). Obtained results were comparable to other recent studies, which revealed that the recent occurrence of carbapenems resistant Gram negative bacteria in neonatal units in different parts of the world is a cause for great concern (Velaphi *et al.*, 2009; Roy *et al.*, 2011 and Lambiase *et al.*, 2012).

Obtained results were relatively in agreement with those found by many investigations in developing country: In Philippines, ampicillin/ sulbactam, gentamicin, and tobramycin showed unacceptably low rates of activity against Gram-negative organisms (Litzow *et al.*, 2009). In India, Gram-negative organisms showed maximum resistant for third generation cephalosporins followed by cotrimoxazole, doxycycline and aminoglycoside combinations of antibiotics ampicillin/sulbactam were sensitive in about 34% of cases.

Table.1 Microorganisms isolated from blood culture of neonatal sepsis patients

Microorganism ³ Groups	Blood sample ¹			
	Male patients(67) No. (%)	Female patients(39) No. (%)	Total male & female ² (106) Total No. (%)	
G+ve bacteria	CONS	24 (35.8)	19(48.7)	43(40.6)
	<i>Micrococcus</i>	9(13.4)	4(10.3)	13(12.3)
	COPS	6(9.0)	4(10.3)	10(9.4)
	<i>Bacillus</i>	4(6.0)	0(0.0)	4(3.8)
	Total	43(64.2)	27(69.2)	70(66.0)
G-ve bacteria	<i>Enterobacter</i>	7(10.4)	4(10.3)	11(10.4)
	<i>Shigella</i>	6(9.0)	1(2.6)	7(6.6)
	<i>E.coli</i>	5(7.5)	0(0)	5(4.7)
	<i>Citrobacter</i>	2(3.0)	2(5.1)	4(3.8)
	<i>Klebsiella</i>	1(1.5)	3(7.7)	4(3.8)
	Total	21(31.3)	10(25.6)	31(29.2)
Yeast & fungi	<i>Candida</i>	3(4.5)	2(5.1)	5(4.7)

1: Collected from Almaza Hospital, Cairo, Egypt, during the period of 12 months (fromDecember2011toDecember2012); 2:The studied newborns (36-76h) were associated with one or more of signs of sepsis; 3: Identified according to Barrow & Feltham(2003); CONS coagulase negative staphylococci; COPS: coagulase positive staphylococci.

Table.2 Resistant 1 pattern of causative late neonatal sepsis

Antibiotic disks (Concentration/disk)	Tested bacteria						
	Gram positive n=53		Gram negative n=31				
	CONS n=43 (%)	COPS n=10 (%)	<i>Enterobacter</i> n=11(%)	<i>Shigella</i> n=7. (%)	<i>E.Coli</i> n=5 (%)	<i>Citrobacter</i> n=4 (%)	<i>Klebsiella</i> n=4 (%)
P (10.0units)	97.7	80.0	ND	ND	ND	ND	ND
AMP (10.0µg)	93.0	70.0	100.0	85.7	100.0	100.0	100.0
AMC (20.0/10.0µg)	76.7	50.0	91.7	71.4	80.0	50.0	100.0
SAM (10.0/10.0µg)	76.7	70.0	83.3	85.7	80.0	50.0	75.0
OX (1.0µg)	79.1	60.0	ND	ND	ND	ND	ND
CPM (30.0µg)	72.1	80.0	66.7	57.1	100.0	50.0	100.0
CTX (30.0µg)	74.4	90.0	66.7	57.1	80.0	75.0	100.0
CA(30.0µg)	74.4	70.0	66.7	85.7	60.0	75.0	75.0
IPM (10.0µg)	30.2	20.0	25.0	71.4	60.0	25.0	50.0
NA (30.0 µg)	69.8	30.0	91.7	42.9	60.0	25.0	50.0
CIP (5.0µg)	51.2	20.0	83.3	42.9	80.0	0.0	50.0
NOR (10.0µg)	39.5	10.0	50.0	14.3	40.0	0.0	50.0
T (30.0µg)	48.8	30.0	33.3	57.1	60.0	50.0	50.0
DA (2.0µg)	60.5	50.0	ND	ND	ND	ND	ND
CLR (15.0µg)	58.1	40.0	ND	ND	ND	ND	ND
CN (10.0µg)	37.2	20.0	58.3	14.3	80.0	50.0	100.0
TPZ (100.0/10.0µg)	55.8	30.0	50.0	28.6	0.0	25.0	25.0
AK (30.0µg)	25.6	10.0	41.7	28.6	20.0	50.0	50.0
LZD(30.0µg)	34.9	30.0	ND	ND	ND	ND	ND
TOB (10.0µg)	25.6	10.0	75.0	71.4	60.0	25.0	75.0
S (10.0µg)	37.2	20.0	75.0	28.6	60.0	50.0	0.0
OFX (5.0µg)	58.1	50.0	75.0	42.9	60.0	50.0	50.0
DO (30.0µg)	44.2	30.0	41.7	28.6	40.0	50.0	50.0
VA(30.0µg)	51.2	70.0	ND	ND	ND	ND	ND

1: by disk diffusion method according to CLSI (2011), n: total number, ND: not determined, CONS: coagulase negative staphylococci; COPS: coagulase positive staphylococci.

Fig.1 Overall antibiotics susceptibility pattern of Staphylococcus isolates

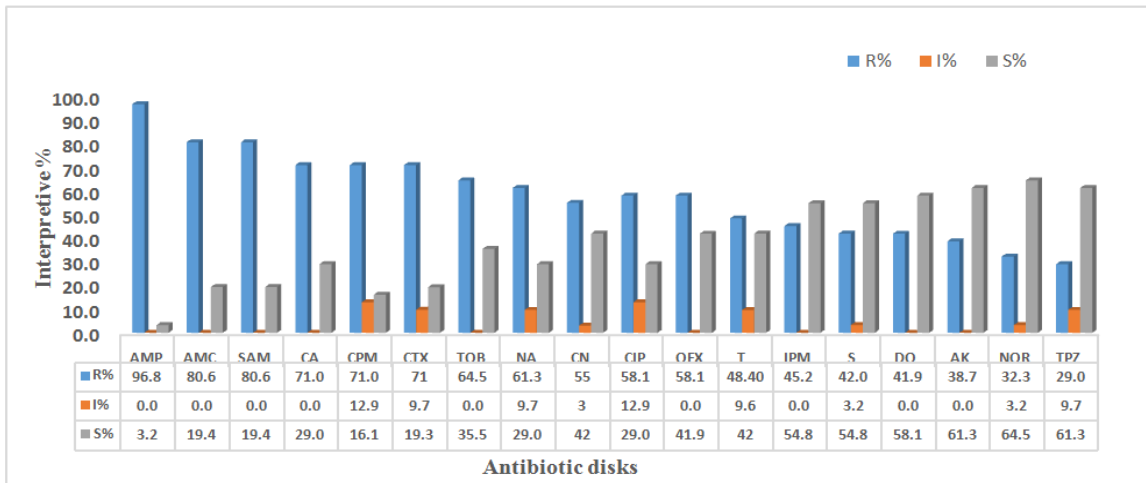


Fig.2 Overall antibiotics susceptibility pattern of enterobacterial isolates

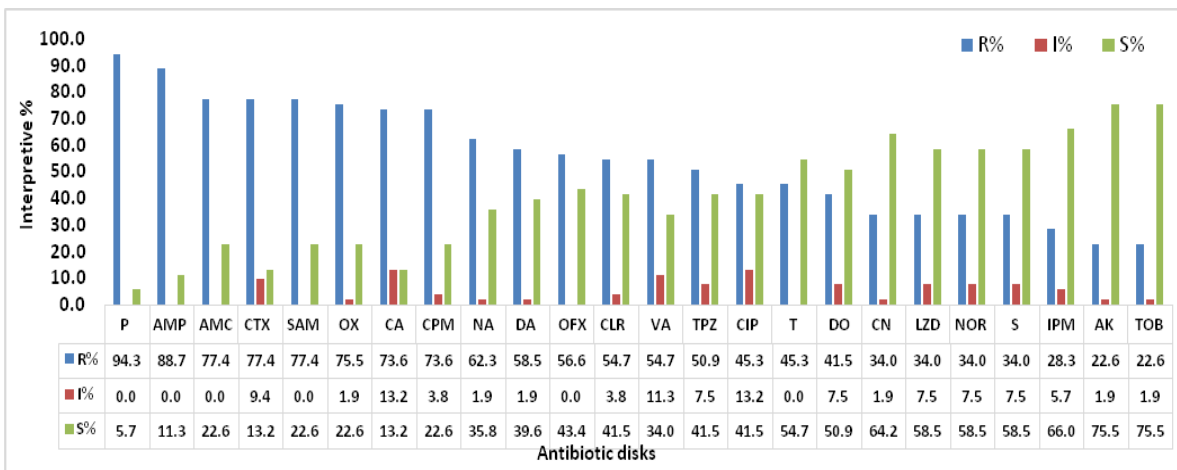


Fig.3 Antibiotic resistance percent of Staphylococcus isolates

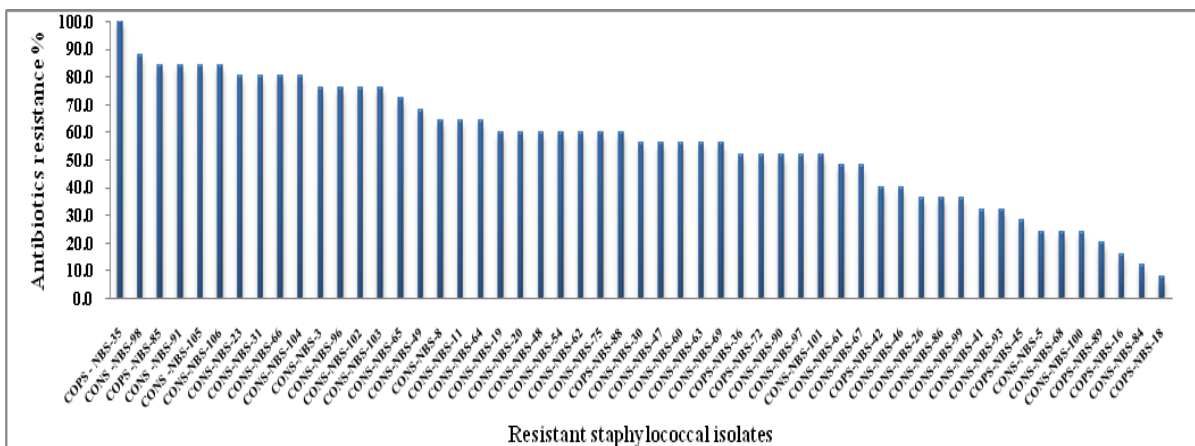


Fig.4 Phylogenetic tree of Staphylococcus-NBS-035

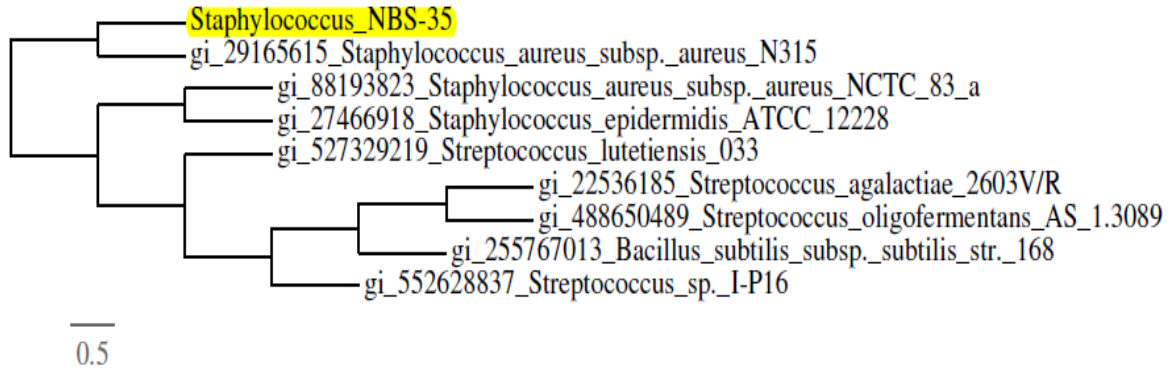


Fig.5 Phylogenetic tree of Staphylococcus-NBS-98

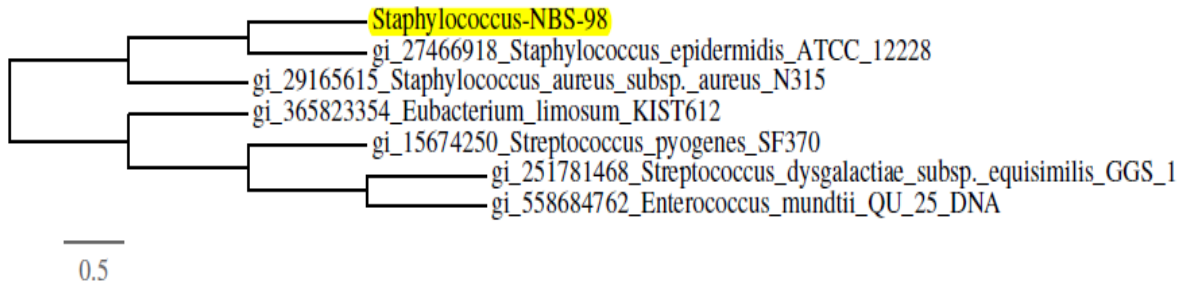


Fig.6 Antibiotic resistance percent of enterobacterial isolates

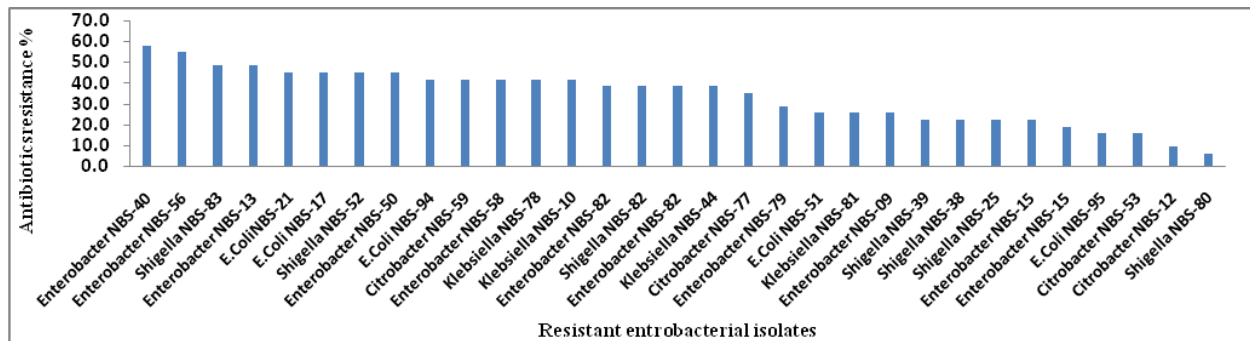
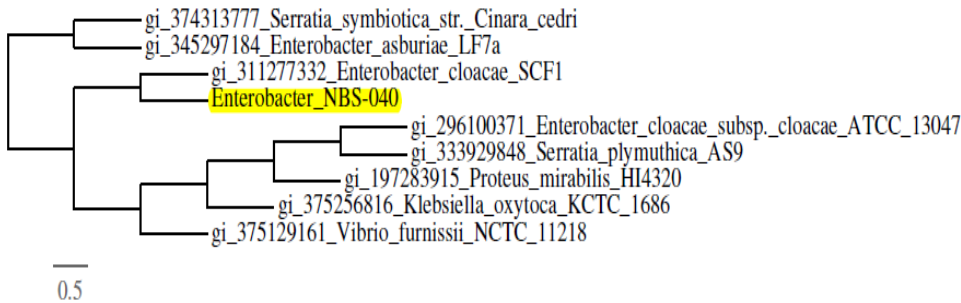


Fig.7 Phylogenetic tree of Enterobacter NBS-40



In addition, 28.6% of Gram negative bacilli were resistant to ceftazidime and 14.3% to aztreonam. In Pakistan, Najeeb *et al.* (2012) reported that in most of the cases causative organisms were found to be resistant to commonly used antibiotics like ampicillin (77.7%), amoxicillin (81.5%), cefotaxime (63.1%) and ceftriaxone (66.9%). There was comparatively less 56.9% resistance to ceftazidime. Gentamicin had resistance in 55.1% cases followed by tobramycin 34.8% and amikacin 17.4%. In Jordan, The most common Gram negative bacteria were Cephalosporin resistant (47 septic episodes), followed by Carbapenems resistant bacteria (28 septic episodes). Of the cases included, 70% were late septic episodes (Al-lawama *et al.*, 2014).

On the other hand, Waheed *et al.* (2003) had found imipenem effective against the some enterobacterial isolates attained to 100%. In our study, high resistance rate may be due to resistant strains of bacteria due to improper use of antibiotics. Imipenem is widely used nowadays and has high sensitivity against both gram-positive and gram-negative bacteria. Shaw *et al.* (2007) had found that 100% sensitivity of imipenem against *Acinetobacter*, *Klebsiella*, *E. coli* and *Enterobacter* species. The present study showed that imipenem sensitivity rate was 57.1%, of which sensitivity against individual bacteria discussed above was 25.8%, 47.1%, 92.35%, 80%, 86.5% and 100% respectively (Najeeb *et al.*, 2012). Rathod *et al.* (2012) reported that, 100% of Gram negative bacilli isolates were sensitive to imipenem and amikacin.

CoNS was the common Gram positive organism, while *Enterobacter* spp. was the most common Gram negative bacilli in LONS. High prevalence of multi-drug resistance among bacteria causing neonatal sepsis is alarming. Thus, continuous

identification of the etiology and surveillance of the bacterial resistance pattern is recommended for effective therapy. Preventive measures should be implemented in hospitals to control LONS. Further work is in progress for identifying effective antibiotic combinations against the most resistant etiological agents.

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