Changing pattern of neonatal bacteremia

Microbiology and antibiotic resistance

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ABSTRACT

Objective: To determine the most important causative agents of bacteremia in a neonatal intensive care unit (NICU); their changing distribution and their antibiotic susceptibility patterns over a 5-year period.

Methods: This study was performed between January 1997 and January 2001 at the Microbiology Section, Tripoli Medical Center, Tripoli, Libya. During this period 1431 Oxoid Signal Blood Cultures sets were obtained from 1092 NICU with suspected bacteremia. Conventional methods, API 20 E and API 20 NE were used to identify the isolated bacteria. The Kirby-Bauer disk diffusion method was performed to assess their antibiotic susceptibilities in accordance with National Committee for Clinical Laboratory Standards.

Results: During the study period, 801 sets out of the total 1431 blood cultures were positive for microbial growth, which represented 648 cases of neonatal bacteremia from a total of 1092 cases. From the total number of isolates, the members of *Enterobacteriaceae Serratia, Klebsiella* and *Enterobacter spp* were the most common cause of bacteremia. The coagulase negative and positive *Staphylococci* were also frequently isolated. A changing pattern of causative pathogen was observed during this study between members of the 3 leading genera of *Enterobacteriaceae.* Antibiotic susceptibility testing showed a high level of resistance among the most common pathogens. Resistance to aztreonam, imipenem, ciprofloxacin and piperacillin/tazobactam was less frequently encountered. *Staphylococcus* resistance to anti-staphylococcal antibiotic and due to hyperproduction of penicillinase enzyme was also high and all isolates were remained sensitive to vancomycin.

Conclusion: Gram-negative bacteria especially members of the *Enterobacteriaceae* are important causes of bacteremia in NICUs. Although most isolates remain sensitive to the new antibiotics, emergence of resistant strains cannot be excluded in the future. For that reason, new strategies and continuous surveillance are required to monitor the changing epidemiology of pathogens, antibiotic susceptibilities and antibiotic use needed to overcome the increasing incidence of resistance to conventional drugs.

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Despite advances in diagnostic facilities and antibiotic chemotherapy, mortality from septicemia has not been reduced significantly compared to the pre-antibiotics era.[1] Bacterial infections continue to be important causes of morbidity and mortality in neonatal patients, although fungal and viral infections have been recognized with increasing frequency.[2] There are several important factors that influence the outcome of bacteremia such as patient age, underlying conditions, microorganism involved and its origin, as well as antibiotic therapy.[3] Changes relating to

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the nature of bacterial infections have occurred over the past 3 decades, these include changes in the spectrum of infections caused by both gram positive and gram negative bacteria,[4] and the antimicrobial susceptibilities of isolates leading to the emergence of several multiple-drug resistant organisms.[5] In this respect, it has become necessary to engage in appropriate epidemiological surveillance to identify the etiological agents and their antibiotics susceptibilities so that the emergence of new pathogens and their resistance patterns can be monitored. The aim of this study was to investigate the most important causative agents of bacteremia in neonatal patients over a 5-year period and to determine the antibiotic susceptibility patterns.

Methods. This was a prospective study carried out in the Microbiology Section, Tripoli Medical Center, Tripoli, Libya on neonates attended the special care baby unit. The annual number of admissions is approximately 763 per year. Mortality rate account for almost 35% of the admissions per year. Babies with a clinical diagnosis of neonatal sepsis and blood culture sent for microbiology analysis were only enrolled in this study.

Blood cultures. A total of 1431 Oxoid Signal Blood Culture sets representing 1092 neonatal patients with diagnosed septicemia from 1997 to 2001 were analyzed and studied. Sample collection and inoculation procedure were performed in accordance with instruction leaflet provided with Oxoid Signal Blood Culture System.

Identification of microorganisms. A total incubation period of at least 7 days was allowed for blood culture bottles before they were discarded as negative. Terminal subculture from negative bottles at the end of incubation period was also performed. Positive culture bottles during incubation period were removed and a sample of blood/broth mixture for subculture, microscopy and susceptibility testing was carried out. Subcultures were made onto chocolate agar, MacConkey agar and 2 blood agar plates. One blood agar was incubated anaerobically and other plates aerobically. All plates were left for up to 7 day before being discarded as negative. The cultures were interpreted according to type of microorganism, frequency of isolation, clinical relevance of isolate and number of episodes when the significance of isolate could not be determined. This was considered when the growth of microorganisms in the bottle was due to normal skin flora from neonates represented by single blood culture isolation. The identification of microorganisms were carried out using conventional methods and the routine procedures such as colony appearance, gram reaction, morphology, and biochemical reactions[6] as well as API 20 E and API 20 NE (bioMerieux, France).

Antibiotic susceptibility. Bacterial isolates were tested for susceptibility to various antimicrobial agents obtained from Oxoid on Mueller Hinton agar using Kirby-Bauer disk diffusion methods in accordance with the National Committee of Clinical Laboratory Standard (NCCLS).[7] The isolates were categorized as sensitive or resistant using the criteria described by NCCLS in the guide to sensitivity testing.[6,7]

Oxacillin resistant. *Staphylococcal* isolates which showed resistance using disk diffusion method were submitted for further investigation to determine whether the resistance was either due to penicillin binding proteins (PBPs) alteration or as a result of hyperproduction of beta-lactamase enzyme which termed as borderline oxacillin-resistance (BMRS/BORS). The test was followed as directed by NCCLS guidlines.[6,8] For each isolate 100 ml of a 0.5 McFarland suspension was streaked on a Muller-Hinton agar plate supplemented with 4% NaCl and 6µg of oxacillin per ml. The plates were then incubated for 48 hours at 35^{o}C. Any growth on the plate was recorded as indicating resistant.[6,8]

Borderline oxacillin resistant. The chromogenic cephalosporin nitromifene disk test was used as recommended by the manufacturer (Oxoid) on each of the staphylococcal strains resistant to oxacillin. Also, these strains were tested for susceptibility to amoxicillin clavulanic acid (20µg and 10µg) and oxacillin disk containing 5µg. By disk diffusion placed 15 mm apart according to NCCLS guidelines. Following incubation for 24 hours at 35^{o}C any enhancement of the zone of inhibitor between oxacillin disk and that containing the beta-lactamase inhibitor was indicative of the presence of hyperproduction of penicillinase enzyme by staphylococcal strains.[8]

Results. During the study period a total of 1431 episodes of blood cultures specimens representing 1092 NICU patients with diagnosed bacteremia were analyzed for culture growth. Out of the total, 801 were found positive, which corresponds to 648 neonates with proved bacteremia (positivity rate of 59.3%). **Table 1** showing a detail of the numbers of neonates, with the reference to episode of blood culture for each as well as number of neonates with bacteremia during the period of this study.

The types of microorganisms, their frequency of isolation in each year and during the 5-years period are shown in **Table 2**. Briefly the gram-negative rods of members of *Enterobacteriaceae* were the most common isolates during each year period and over the whole 5-years of the study. Furthermore, changing patterns of etiological agents have also been noted from one year to another. This is can be noted by the replacement of *Serratia spp,* which was the leading pathogen during the years 1997 and

Table 1 - Represent the detailed of blood cultures result according to number of episodes submitted for microbiological culture.

N of episodes	N of neonatal	Female	Male	Total blood cultures	Positive	Negative	Positive %	N of neonatal with positive growth
1	823	759	64	823	440	383	53.5	440
2	215	200	15	430	238	192	55.3	159
3	44	42	2	132	85	47	64.4	40
4	5	5	0	20	15	5	75.0	4
5	4	4	0	20	17	3	85.0	4
6	1	1	0	6	6	0	100.0	1
Total	**1092**	**1011**	**81**	**1431**	**801**	**630**	**56.0**	**648**

Table 2 - Microorganisms isolated from neonatal blood cultures with reference to their frequency number during 5 years period.

Microorganism	1997	1998	1999	2000	2001	Five years
Serratia Marcescens	105*	103	1	0	1	210
Serratia liquefaciens	65	57	7	4	11	144
Klebsiella pneumoniae	26	4	30	88	13	161
Enterobacter cloacae	26	14	2	8	7	57
Enterobacter agglomerans	2	0	1	10	59	72
Staphylococcus epidermidis	19	5	8	9	11	52
Staphylococcus aureus	0	2	5	9	9	25
Pseudomonas species	7	3	2	3	10	25
Other negative rods	9	5	3	11	9	37
Other positive cocci	4	2	1	5	4	16
Candida albicans	2	0	0	0	0	2
Total number	**265**	**195**	**60**	**147**	**134**	**801**

Table 3 - Susceptibility of common leading gram negative rod pathogen to various antibiotics.

Antibiotic	*Serratia marcescens* n (%)	*Serratia liquefaciens* n (%)	*Klebsiella pneumoniae* n (%)	*Enterobacter cloacae* n (%)	*Enterobacter agglomerans* n (%)
Ticarcillin/clavulanic acid	210 (98)	144 (100)	161 (100)	48 (83)	63 (90)
Cefuroxime	210 (100)	144 (100)	161 (100)	58 (100)	67 (96)
Cefoperazone	210 (100)	144 (100)	161 (100)	58 (100)	65 (93)
Cephaloridine	210 (100)	144 (100)	161 (100)	58 (100)	70 (100)
Ceftriaxone	210 (100)	144 (100)	161 (100)	23 (40)	15 (21)
Carbenicillin	210 (100)	144 (100)	161 (100)	58 (100)	70 (100)
Ampicillin	210 (100)	144 (100)	161 (100)	58 (100)	70 (100)
Amoxicillin	210 (100)	144 (100)	161 (100)	58 (100)	70 (100)
Amoxicillin/clavulanic acid	210 (100)	144 (100)	157 (98)	58 (100)	70 (100)
Gentamicin	201 (96)	136 (94)	153 (95)	50 (86)	63 (90)
Sulfamethoxazole/trimethoprim	62 (30)	43 (30)	133 (83)	43 (74)	31 (44)
Amikacin	84 (40)	57 (40)	100 (62)	24 (41)	28 (40)
Ceftazidime	24 (11)	19 (13)	97 (60)	20 (34)	18 (26)
Piperacillin/tazobactam	91 (43)	63 (44)	42 (26)	0 (0)	6 (9)
Doxycycline	177 (84)	122 (85)	42 (26)	25 (43)	34 (49)
Ciprofloxacin	6 (3)	5 (3)	6 (4)	0 (0)	0 (0)
Aztreonam	29 (14)	27 (19)	5 (3)	17 (29)	9 (13)
Imipenem	6 (3)	6 (4)	0 (0)	4 (7)	5 (7)
Total isolates tested	**210**	**144**	**161**	**57**	**72**

1998 by *Klebsiella pneumoniae* in the year 1999 and 2000. During the year 2001 both species were replaced by *Enterobacter agglomerans*. Results for the total period show that the 4 genera of *Enterobacteriaceae* are the predominant causes of bacteremia in neonates. Other gram-negative rods less commonly isolated are *Escherichia coli* (*E. coli*) (7 isolates), *K. oxytoacae* (4 isolates), *Enterobacter aerogenes* and *Enterobacter sakazakii* (3 isolates each), *Enterobacter intermedius*, *Citrobacter freundii* and *Serratia polymathic* (1 isolate each). Non-fermented gram-negative bacteria isolated are *Acinetobacter spp* (17 isolates) in which *Acinetobacter baumannii* (11 isolates) and *Acinetobacter calcoaceticus var anitratus* (6 isolates) were found. *Pseudomonas spp* (25 isolates) in which (12 isolates) *Pseudomonas aeruginosa*, *Pseudomonas spp* (12 isolates) and Pseudomonas CDC Group (I isolate) were isolated. Among gram positive bacteria *Staphylococcus epidermidis* and *Staphylococcus aureus* are the most frequently isolated species. Other positive cocci isolated are *Beta-haemolytic streptococci* (6 isolates), *Alpha-streptococci* (2 isolates) and *Streptococcus pneumoniae* (1 isolate).

The results of antibiotic susceptibility testing for the most important gram negative pathogens are shown in **Table 3**. Almost all isolates of the *Enterobacteriaceae* were either completely resistant or showed sensitivity to early beta-lactam antibiotics including ampicillin, amoxicillin, carbenicillin alone or in combination with beta-lactam inhibitor clavulanic acid. Furthermore, these isolates were also resistant to second generation cephalosporin and to some extent to third generation drugs such as ceftriaxone. Resistance to other antibiotics including gentamicin, amikacin and sulfamethoxazole/trimethoprim was also high. Although the broad beta-lactam antibiotics including aztreonam, ceftazidime, imipenem and piperacillin/tazobactam were consider to be effective against these microorganism a good number of isolates were found to be resistant to them. This finding was noted with ceftazidime, which rise the question to the possibility for emergence of extended-spectrum beta-lactamase resistance (ESBLR).

Imipenem and ciprofloxacin were the only antibiotics showing good effect against all isolates. Among gram-positive cocci tested isolates (**Table 4**) both *Staphylococcus aureus* and *Staphylococcus epidermidis* showed high resistance toward penicillins including resistance to oxacillin when tested by disc diffusion method. However, when these resistant strains were submitted for further studies for confirmation it was found that of the total isolates tested of *Staphylococcus aureus* (15 isolates) and *Staphylococcus epidermidis* (38 isolates) showed resistance to oxacillin using disc diffusion method. The re-testing of these isolates showed that *Staphylococcus aureus* from the 15 isolates were resistant to oxacillin, 3 isolates were only found to be truly resistant to oxacillin while 12 isolates were hyper-producing strains (**Table 4**). Regarding *Staphylococcus epidermidis* of the total, 38 isolates showed resistance to oxacillin by disc diffusion only 19 isolates were found to be true oxacillin resistant and 19 isolates were hyper-producing stains. There was a very high rate of beta-lactamase positivity among all staphylococcus strains.

Resistance of both isolates to other antibiotics including members of macrolides, aminoglycosides, sulfamethoxazole, doxycycline and fusidic acid was also high. The best observed results against both isolates were only noted with imipenem, vancomycin and ciprofloxacin.

Table 4 - Susceptibility of *Staphylococcus spp* to various antibiotics.

Antibiotic	***Staphylococcus aureus*** **n (%)**	***Staphylococcus epidermidis*** **n (%)**
Penicillin G	25 (100)	51 (98)
Fusidic acid	23 (92)	44 (85)
Amoxicillin	22 (88)	39 (75)
Ampicillin	21 (84)	42 (81)
Erythromycin	21 (84)	37 (71)
Sulfamethoxazole/trimethoprim	18 (72)	20 (38)
Lincomycin	17 (68)	38 (73)
Doxycycline hydrochloride	17 (68)	37 (71)
Oxacillin	3 (12)	19 (37)
Borderline oxacillin resistant	12 (48)	19 (37)
Gentamicin	12 (48)	38 (73)
Cephaloridine	8 (32)	20 (38)
Amoxicillin/clavulanic acid	7 (28)	20 (38)
Imipenem	0 (0)	10 (19)
Ciprofloxacin	0 (0)	7 (13)
Vancomycin	0 (0)	0 (0)
Total isolate tested	**25**	**52**

Discussion. Tripoli Medical Center is a modern recently built hospital opened in September 1996, with a capacity of 1438-beds. It also includes out patient's clinics and provides free health care services for Libyan nationals. Furthermore, it is consider being one of the largest teaching hospital for medical students in the country.

Approximately 5 million neonatal death occur world wide every year, 98% of which occur in developing countries, particularly Africa and Asia. Infections such as septicemia, pneumonia, meningitis and diarrhea account for 30-50% of neonatal deaths in developing countries.[9] Neonatal bacteremia is a life threatening emergency and delay in treatment may result in death.[2]

The data in this prospective study represent the first report analyzing blood cultures of neonatal patients in our hospital setting. The spectrum of organisms causing neonatal bacteremia in our study is similar to that reported for other neonatal units in developing countries, with the gram negative organisms being responsible for most cases.[9] The data shows clearly that for most cases of neonatal bacteremia (823) only one set of blood collection was sent for microbiological investigation. This may be due to difficulties in obtaining more than one blood sample during a 24 to 48 hours' period as recommended when using this system. Since a positive result of a blood culture from bacteremic patient will depend on the number of organisms in the blood at the time of collection and the numbers and volume of the sample. This is may explain the decrease in positive growth rate from neonates representing with single blood culture comparing to patients representing with more than one episode (**Table 1**). Such finding emphasizes the need to advice and encourage physicians to send more than one blood culture to confirm bacteremia in neonates.[10]

Gram-negative bacilli were found to be the major cause of bacteremia in neonates especially most the important members of *Enterobacteriaceae*. The predominant of these organisms in neonates in hospital may be due to the fact that most of these are normal commensals and widely distributed in hospital environment. Lack of basic facilities and practices such as hand washing and lack of surveillance of the standards maternity homes may attributed to the increasing incidence of bacteremia in neonate unit. Also, the successes of antibiotics in controlling many gram-positive infections and advances in medical technology promoted the selection of gram-negative rods in the hospital environment.[11] Our results show clear change in bacteria causing bacteremia during the 5-years period within the 3 member's genera of *Enterobacteriaceae* representing by *Serratia*, *Klebsiella* and *Enterobacter species* and they remained as important causes of bacteremia among neonates over the period. A similar findings were reported by others worldwide.[9] Antibiotics susceptibility of leading pathogens in this study presented in **Table 3** found them to be either completely insusceptibility to penicillins antibiotics which exert an antimicrobial effect against such bacteria. Including some of third generation Cephalosporin or shifting toward an emerging increase in strains resistant to new broad spectrum beta-lactam antibiotics such as ceftazidime. It is well documented that the indiscriminate use of antibiotics has led to the selection and dissemination of antibiotics-resistant organisms. Several authors have reported the association of resistance to beta-lactam antibiotics with prior use to third generation-cephalosporins. A common mechanism of resistance to cephalosporins and other beta-lactam antibiotics among *Klebsiella spp* and other members of *Enterobacteriaceae* is the production of ESBL. This probably explain the increasing incidence of resistance of *Klebsiella* to ceftazidime and early beta-lactam antibiotics in our hospital setting. This finding was not surpassingly in our study, since a number of surveillance studies reported an increasing rate of resistant strains among member of *Enterobacteriaceae* including *Klebsiella, Serratia, Proteus, E. coli* and *Enterobacter species* throughout the world.[12] This emerging resistance represents a major threat to successful therapy of nosocomial infections.[13] The increased use of third generation cephalosporin, other broad-spectrum antibiotics and the lack of Infection Control Committee, probably led to the selection of resistant strains among these bacteria which became frequently resistant to multiple antibiotics including many of beta-lactam antibiotics and aminoglycosides. The high numbers of antimicrobial resistant gram-negative bacilli in our study strongly suggested multiple mechanisms of antimicrobial resistance including the presence of numerous extended spectrum and chromosomally derepressed beta-lactamase to all commercially available beta-lactam exist now. However, although there are some increase in numbers of resistant strains of the leading pathogens toward broad-spectrum beta-lactam antibiotics, these drugs particularly, aztreonam, imipenem, piperacillin/ tazobactam and ciprofloxacin, still show high activity overall.

Among gram-positive bacteria *Staphylococcus epidermidis* and *Staphylococcus aureus* are found to be the most important cause of bacteremia in neonates in our study after the leading members of *Enterobacteriaceae*. Several studies have revealed that the proportion of cases of bacteremia in intensive care units and neonatal units caused by negative *Staphylococcus* and coagulase positive increased dramatically in the last few years.[14] The increased use of invasive devices, such as catheters, and expanding numbers of patients with impaired host defenses as well as the emergence of resistance to anti-gram positive agents have contributed to the occurrence of infections due to *Staphylococcus spp*. This is found to be in our study, which demonstrated a high level of resistance of *Staphylococcus spp* to the most anti-*Staphylococcu*s drugs. The true incidence and discrepancies between phenotypic and genotypic resistant of methicillin and oxacillin resistance was clarified by re-testing all *Staphylococcus* isolates, which showed either resistance or reduced susceptibility to methicillin and oxacillin, by the disc diffusion method. The findings clearly indicate that oxacillin resistant *Staphylococcus aureus* in our study is very low in

which, resistant appears to be as a result of hyper production of penicillinase enzyme by these strains. This usually renders them either resistant or of reduced susceptibility by disc diffusion method.[15] This is confirmed by the fact that the susceptibility was restored to normal when the drug was combined with beta-lactamase inhibitor (clavulanic acid). However, oxacillin resistance in *Staphylococcus epidermidis* were found to be high as well due to hyperproduction of penicillinase enzyme, in which they were found to account for approximately 50% of all tested isolates (**Table 4**). Recently strains of *Staphylococcus aureus* and *Staphylococcus epidermidis* demonstrated reduced susceptibility to anti-staphylococcal penicillins, the activity of were restored when combined with beta-lactamase inhibitors.[16] Such strains, termed "borderline oxacillin-resistant" have been noted to be hyperproduces of beta-lactamase.[17] Since there was such a high rate of beta-lactamase production in most of *Staphylococcus* strains, as may be expected, resistance to penicillin, ampicillin, amoxicillin in both the oxacillin sensitive and oxacillin resistant was high. Strains variation in the level of beta-lactamase production may also attributed to the finding that some were showed susceptibility to these antibiotics. Although some oxacillin resistant strains showed to be susceptibility to imipenem, these strains should be considered reported resistant to all beta-lactam antibiotics including imipenem. Vancomycin was found still to be the drug of choice for treating serious infections due to the presence of oxacillin resistant *Staphylococcus*. Finally, continual surveillance of prevalent strains and their resistant patterns is fundamental as a means of establishing the significance of resistance in clinical infection, and in the determination of hospital prescribing policies. Antibiotic resistance surveillance programs are necessary to promote optimal use of antibiotics. Proper infection control procedures must also be practiced to prevent horizontal transfer of drug-resistant organisms.

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