A multi centre laboratory study of Gram negative bacterial blood stream infections in Sri Lanka

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(Index words: antibiotic sensitivity, sepsis, antibiotic resistance)

Abstract

Introduction Data on causative agents and antibiotic susceptibility patterns of blood stream infections in Sri Lanka is scarce. Information on trends of antibiotic resistance is necessary for the prescribers to treat patients effectively and policy makers to develop policies and guidelines.

Objectives To lay the foundation for a national data base on antimicrobial resistance in Sri Lanka.

Methods A prospective study was carried out in seven hospitals to study the Gram negative aetiological agents and their susceptibility patterns in patients suspected of having bacteraemia. We reviewed 817 patients with clinically significant blood cultures including both adults and children.

Results Data were complete for analysis in 733 Gram negative isolates only. Of the 733 isolates, 488 were from adults (> 12 years), 109 were from children (1-12 years) and 136 were from infants (<1 year). Intensive care units represented 18.4% of the isolates (123 adult patients and 27 paediatric patients). The highest number of isolates (33.7%) was from patients with septicaemia of unknown origin. Enteric fever, pyelonephritis and respiratory tract infections accounted for 20% of the isolates. Bacteraemia with underline malignancies were responsible for 24.5% of infections. *Salmonella paratyphi* A was the commonest cause of enteric fever in adults with 92% resistance to ciprofloxacin. The prevalence of extended spectrum beta lactamase (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* was high in this study population.

Conclusions It is essential to introduce multidisciplinary interventions to reduce the inappropriate use of antibiotics to increase the lifespan of precious antibiotics. Introduction of a National antibiotic policy with strict implementation and a well-planned stewardship programme is essential to control antimicrobial resistance in our country.

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Introduction

Antimicrobial resistance is a serious problem worldwide. Emergence and rapid spread of resistance is common in developing countries with uncontrolled antibiotic usage and overcrowding of health care facilities [1, 2]. Correct empirical antibiotic therapy plays a critical role in successful outcome of Gram negative sepsis and can save many lives by timely intervention.

In Sri Lanka there was no existing system to gather information on antibiotic resistance on a multicenter basis [3]. This long felt need was addressed by the Sri Lanka College of Microbiologists by carrying out this multicentre study on aetiological agents and antibiotic resistance. This study was initiated to determine the prevalence of Gram negative pathogens causing bacteraemia and to determine their sensitivity patterns according to clinical diagnosis. The main objective of this project was to lay the foundation for a national data base on antimicrobial resistance in Sri Lanka. Evidence shows that surveillance, when used to guide policies on antibiotic use and infection control, can be helpful in the fight to control the development and spread of resistance [4].

The project was planned in three phases. This report describes the data from phase 1 of the project which include Gram negative organisms isolated from blood cultures and their resistance patterns.

Methods

A prospective study was conducted in seven centres namely, National Hospital of Sri Lanka, Colombo South Teaching Hospital, National Cancer Institute, Medical Research Institute, Teaching Hospital, Kandy, Provincial General Hospital, Ratnapura and Faculty of Medicine, Colombo, from March 2009 to February 2010.

The significance of Gram negative isolates in a blood culture to be included in this study was decided by the microbiologists in respective centres. Isolates were considered as significant when taken from patients with high fever with a matching provisional diagnosis, a Gram negative isolate from 24-72 hours of incubation, a pure growth of isolate of the same patient with the same organism from more than one blood culture, *Salmonella* spp. isolated from blood irrespective of duration of incubation period and an isolate from peripheral and central venous catheters with the same organism. From a patient with multiple blood culture isolates only the first isolate was considered for analysis. Blood cultures with mixed growths, isolates only from central venous lines or of doubtful clinical significance were rejected.

Blood cultures were processed either by manual method or by automated blood culture systems using the standard microbiological methods. Identification of

organisms was done using Gram stain, API 20E, API 20NE and by serotyping. Antibiotic susceptibility testing was carried out by CLSI method [5]. Results obtained were analysed by demographic and clinical data of the patient and the sensitivity of the selected pathogens. According to the CLSI guideline only species with testing data for thirty or more isolates were used for analysis of cumulative antibiogram [6]. Therefore 223 isolates belonging to 66 species were not analysed for susceptibility patterns as the numbers were too small.

Results

The surveillance sample included information on 817 Gram negative isolates. Data were complete for analysis in 733 isolates only. Of these 733 isolates, 488 were from adults (> 12 years), 109 were from children (1-12years) and 136 were from infants (<1 year). Intensive care units represented 18.4% of total isolates. This comprised of 123 adult patients and 27 paediatric patients.

It was not possible to determine the origin of sepsis in many of the culture positive bacteraemias (33.7%). Enteric fever contributed to 10.2% of bacteraemias while pyelonephritis (4.5%) and respiratory tract infections (4.5%) contributed equally. Sepsis due to invasive procedures contributed to 2.3% of the infections while sepsis from wound infections was 1.5%. In adults the percentage of bacteraemia due to enteric fever, pyelonephritis and invasive procedures was higher than in children. Infective endocarditis contributed to bacteraemia in less than 1% (Table1). Haematological illnesses, especially leukaemia was the second most frequent (15%) clinical condition which gave positive blood cultures. Solid tumours contributed to 9.7% of bacteremias.

Table 1. Sources of infection in blood culture positive patients

Origin of sepsis	*Frequency*	*%*
Septicaemia of unknown origin	275	33.7
Infections associated with haematological illnesses	119	14.6
Enteric fever	83	10.2
Malignancy (solid tumour) related infections	81	9.9
Pyelonephritis	37	4.5
Respiratory infections	37	4.5
Central nervous system infections	30	3.7
Gastro intestinal infections	23	2.8
Infections due to invasive procedures	19	2.3
Sepsis due to surgical wound infection	12	1.5
Deep seated abscesses	7	0.9
Infective endocarditis	7	0.8
Gangrene	3	0.4
Septic abortion	1	0.1
Not recorded	83	10.2
Total	817	100

In children, infections associated with haematological illnesses such as leukaemia and aplastic anaemia were the commonest cause (50.5%) while solid tumour malignancies was the second commonest (14.7%) cause of bacteraemia. Among 12.8 % of children, the origin of septicaemia was unknown. None of the children had bacteraemia due to invasive procedures. Enteric fever was the cause in 6.4% of children.

Among infants, origin of septicaemia was not known in the majority (77.9%). Out of the known causes, central nervous infections was the commonest (8.8%). Respiratory infections (4.4%), invasive procedures (2.2%) and haematological malignancies (2.2%) were the next common causes for sepsis in infants.

Aetiological agents

In adult patients having sepsis of unknown origin, the commonest Gram negative organism isolated was *Escherichia coli.* ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* accounted for 22.5% of total infections. (12.6% *Escherichia coli* and 9.9% *Klebsiella pneumonia). Pseudomonas aeruginosa* was the causative agent in 10.6% of infections. *Acinetobacter baumannii calcoaceticus* was encountered in 8.6% of the infections. Majority of isolates in children were non fermenters (64.3%). Of that *Acinetobacte rbaumannii calcoaceticus* accounted for 21.4% followed by *Burkholderia cepacia and Psudomonas aeruginosa* (14.3% each). Non ESBL producing *Klebsiella pneumoniae ssp. Pneumoniae* caused 14.3% of infections in children. Among infants, 52.43% of the isolates were due to ESBL producers followed by non ESBL producing *Escherichia coli* (10.38%.). *Acinetobacter spp.* and *Enterobacter spp.* accounted for 15% of infections.

Out of the enteric fever causing organisms *Salmonella paratyphi* A was the predominant serotype isolated from adults (86.0%) of which 91.6% were ciprofloxacin resistant while in children the predominant serotype was *Salmonella typhi* (85.7%).

In adults with solid tumours nearly 1/6th of infections were due to ESBL producing *Escherichia coli* (18.3%) followed by ESBL producing *Klebsiellapneumoniae* (8.3%). *Enterobacter cloacae* also accounted for 8.3% of infections. In adults with haematological malignancies, the commonest pathogen isolated was ESBL producing *Escherichia coli* (14.8%) and the second commonest was non ESBL producing *Escherichia coli* (11.5%). *Acinetobacter baumannii calcoaceticus* accounted for 18.2% of infections in children with solid tumour and haematological malignancies. *Burkholderia cepacia* and ESBL producing *Escherichia coli* each accounted for 10.9% of isolates in children. In all patients with solid tumours *Acinetobacter baumannii calcoaceticus* accounted for 31.3% infections.

In pyelonephritis 47.5% of the isolates were *Escherichia coli.* Majority was due to non ESBL producing

Escherichia coli. ESBL producing organisms accounted for 30% of infections.

Pseudomonas aeruginosa accounted for 20% of respiratory tract infections followed by *Acinetobacter baumannii calcoaceticus* and non ESBL producing *Escherichial coli* (12% each). Majority of isolates were multidrug resistant with multidrug resistant *Acinetobacter* spp accounting for 50%.

Escherichia coli accounted for 39.1% of intra abdominal infections. Of them 21.7% were ESBL producers.

Acinetobacter baumannii calcoaceticus, Pseudomonas aeruginosa and *Burkholderiacepacia* accounted for 70% of the isolates related to infections due to invasive procedures. *Klebsiella pneumoniae ssp. Pneumoniae* contributed to 13.3%. In adults multi resistant *Acinetobacter* spp accounted for 50% of isolates.

ESBL producers accounted for 10% of infections in the central nervous system of children. Most of these patients were from neurosurgical units.

The total number of isolates from ICUs was 150. *Klebsiella* species producing ESBL accounted for 18.7% while 14% was due to *Pseudomonas aeuruginosa*. ESBL producing *Klebsiella spp.* and *Escherichia coli* together accounted for 26%.

Antibiotic sensitivity patterns

Acinetobacter baumannii calcoaceticus, showed only 60% and 55.6% sensitivity to meropenem and imipenem. The highest sensitivity was to amikacin (69.5%).

Pseudomonas aeruginosa showed more than 50% susceptibility to the antibiotics tested with the highest sensitivity (90%) for piperacillin – tazobactam. Among aminoglycosides, the highest sensitivity was amikacin. Sensitivity to imipenem and meropenem were 69.2% and 72.7%. *Burkholderia cepacia* showed more than 75% sensitivity to ceftazidime, meropenem and cotrimoxazole (Table 3).

Majority of the ESBL producing *Escherichia coli* isolates were from patients with malignancies (39%) and from ICUs (23%). All *Klebsiella pneumoniae ssp. pneumoniae* isolates showed 100% sensitivity to carbapenems and more than 80% sensitivity to amikacin. A high level of resistance was seen against gentamicin (Table 4).

Table 2. Gram negative organisms isolated in blood stream infections

Organisms	*%*
Klebsiellapneumoniae ssp. pneumoniae (ESBL)	13.09
Acinetobacter	11.16
Escherichia coli 1(ESBL)	10.06
Salmonella paratyphi A	10.06
Pseudomonas	8.95
Escherichia coli 1 (Non ESBL)	8.68
Enterobacter	7.99
Klebsiellapneumoniae ssp. pneumoniae (Non ESBL)	6.33
Burkholderia	4.27
Salmonella typhi	2.62
Salmonella spp.(except typhi and paratyphy)	1.79
Proteus	1.52
Other microorganisms isolated (40 species)	13.50

Table 3. Antibiotic susceptibility patterns of non-fermenters

	Acinetobacterspp(n=71)		*Pseudomonas spp(n=48)*		*Burkholderiacepacia n=26)*	
Antibiotic	*Sensitive No./ Total tested*	*% Sensitive*	*Sensitive No./ Total tested*	*% Sensitive*	*Sensitive No./ Total tested*	*% Sensitive*
Amikacin	41/59	69.5	31/41	75.6		
Ceftazidime	26/57	45.6	25/38	65.8	19/25	76.0
Ciprofloxacin	31/46	67.4	22/25	88.0		
Gentamicin	32/61	52.5	25/39	64.1		
Cefepime	23/49	46.9	18/26	69.2		
Imipenem	25/45	55.6	18/26	69.2		
Meropenem	24/40	60.0	16/22	72.7	18/19	95.4
Trimethoprim-sulfamethoxazole	7/13	53.8			6/7	85.7
Ticarcillin-clavulanic acid	7/15	46.7	9/13	69.2		
Piperacillin-tazobactam	26/44	59.1	18/20	90.0		

Non-ESBL producing *Escherichia coli* had 100% sensitivity for carbepenems. More than 90% sensitivity was shown for amikacin, netilmicin and cefepime. Less than 50% sensitivity (16.67%) was seen only to ampicillin. *Klebsiella pneumoniae ssp. pneumoniae isolates*, (non ESBL producing) showed 100% sensitivity to carbapenems. There was 90% sensitivity to piperacillin-tazobactam. More than 80% sensitivity was shown for amikacin, cefepime and netilmicin (Table 5).

A high level of ciprofloxacin resistance was seen among *Salmonella paratyphi A* islolates. Ciprofloxacin resistance was 50% in *Salmonella typhi* (Table 6).

The survival rate for patients who had positive blood cultures were 63.8%, 85% and 65.4% for adults, children and infants respectively. The total number of deaths related to sepsis was 142 (17.4%) and it was lowest among children (5.3%) followed by infants (17.6%) and adults (20.1%). Mortality rate for patients with septicaemia of unknown origin was 47.2%. The survival rate of ICU patients was 60%.

Table 4. Antibiotic susceptibility of ESBL producing *Escherichia coli* and *Klebsiella pneumoninae ssp. pneumoniae*

	Escherichia coli (ESBL) (n=74)		*Klebsiella spp (ESBL) (n=105)*	
Antibiotic	*Sensitive No./ Total tested*	*% Sensitive*	*Sensitive No./ Total tested*	*% Sensitive*
Amikacin	56/67	83.6	79/97	81.4
Ciprofloxacin	10/57	17.5	35/80	43.8
Gentamicin	28/71	39.4	14/101	13.9
Imipenem	54/54	100.0	56/56	100.0
Meropenem	62/62	100.0	70/70	100.0
Netilmicin	41/61	67.2	64/97	66.0
Trimethoprim-sulfamethoxazole	9/59	15.25	11/87	12.6

Table 5. Antibiotic susceptibility of non ESBL producing *Escherichia coli* and *Klebsiella pneumoninae ssp. pneumoniae*

	Escherichia coli (non ESBL)(n=68)		*Klebsiella spp. (non ESBL)(n=39)*	
Antibiotic	*Sensitive No./ Total tested*	*% Sensitive*	*Sensitive No./ Total tested*	*% Sensitive*
Amikacin	53/55	96.36	29/37	85.3
Ampicillin	9/54	16.67	0/22	0.0
Amoxicillin-clavulanic acid	32/52	61.54	16/33	48.5
Ceftazidime	48/55	87.27	20/35	57.1
Ciprofloxacin	32/45	71.11	18/24	75.00
Gentamicin	52/63	82.54	24/34	70.60
Cefotaxime	50/61	82.00	17/32	53.10
Cefuroxime	37/49	75.51	13/26	50.00
Cefepime	38/42	90.48	16/19	84.20
Imipenem	42/42	100.0	22/22	100.00
Meropenem	42/42	100.0	20/20	100.00
Netilmicin	51/56	91.07	28/33	84.80
Trimethoprim-sulfamethoxazole	34/51	66.67	18/31	58.10
Ticarcillin-clavulanic acid	26/34	76.47	12/17	70.60
Piperacillin-tazobactam	35/40	87.50	22/23	95.70

Table 6. Antibiotic susceptibility of *Salmonella paratyphi A* and *Salmonella typhi*

	Salmonella Paratyphi A(n=73)		*Salmonella typhi (n=19)*	
Antibiotic	*Sensitive No./ Total tested*	*% Sensitive*	*Sensitive No./ Total tested*	*% Sensitive*
Ampicillin	52/67	77.6	13/19	68.4
Chloramphenicol	44/44	100.0	13/18	72.2
Ciprofloxacin	6/71	8.4	9/18	50.0
Ceftriaxone	62/63	98.4	19/19	100.0
Trimethoprim-sulfamethoxazole	49/49	100.0	12/19	63.1

Discussion

This surveillance highlights the magnitude of the problem of antimicrobial resistance in Gram negative bacteria in Sri Lanka. In health-care settings, multi-drug resistance in gram negative infections has severely restricted therapeutic options, and sometimes no effective drugs are available to treat life-threatening infections [7]. The isolation of NDM 1 strain in India which produces metallo-beta-lactamases capable of degrading carbepenems indicates the extent of this growing problem [8]. Our data show high prevalence of ESBL producing *Escherichia coli* and *Klebsiella* species. ESBL producing *Escherichia coli* was the commonest pathogen in both solid and haematological malignancies. *Klebsiella pneumoniae* strains harbouring extended spectrum beta-lactamases (ESBL) and more recently metallocarbapenemase, conferring resistance to many of the antibiotics available, have been described in many parts of the world [9]. The available therapeutic options for the treatment of ESBL-associated infections are limited due to co-resistance to various antibiotic classes, including cephamycins, fluoroquinolones, aminoglycosides, tetracyclines and cotrimoxazole [10]. Carbapenems, an antibiotic class that represents the last available weapon against many gram-negative bacilli, are being used increasingly as empirical therapy [7]. In this background there is a high possibility that the unwarranted use of carbepenems will lead to emergence of carbepenem resistant strains such as NDM 1 strains in Sri Lanka too.

According to SENTRY data, ciprofloxacin resistance in enterobacteriaceae in some participating countries was amongst the highest in the world. Over 30% of *Klebsiella pneumoniae* isolates from patients with blood stream infection from mainland China, Philippines, Singapore and South Africa were ESBL producers [11].

The source of sepsis was unknown in 33.7% of patients and the highest mortality rate (47.2%) was seen in patients of this category. In 22.5% of adults and 52.43% of infants, these infections were due to ESBL producing organisms whereas in children, majority of the isolates were non fermenters (64.3%). When considering the pathogens and their antibiotic sensitivity pattern it is apparent that, many of these infections are hospital acquired. Furthermore, ESBL producing *Klebsiella spp.* and *Escherichia coli* were responsible for 26% of septic patients in the ICUs. All these factors highlights and point towards the importance of strengthening the infection control practices in hospitals. The microbial spectrum of uncomplicated cystitis and pyelonephritis consists mainly of *Escherichia coli* (75%-95%), with occasional other species of Enterobacteriaceae [12]. In this study *Escherichia coli* accounted for only 47.5% of pyelonephritis with 30% of isolates being ESBL producers.

In infections due to invasive procedures in adults, multi resistant, non-fermenter *Acinetobacter spp.* accounted for 50% of isolates. Most of these patients were from ICUs. The percentage of deaths in ICU patients was high (40%) as many of these infections were caused by multidrug resistant organisms. According to the surveillance report of European Union, microorganism acquired in ICUs in Europe also has shown a high proportion of resistance [13].

In this study 98.7% of *Salmonella paratyphi A* was resistant to ciprofloxacin as confirmed by previous studies [14, 15]. Unrestricted and unwarranted prescribing of ciprofloxacin in the community may have lead this. Although Salmonella isolates were relatively sensitive to ceftriaxone in this study, there have been sporadic reports of high-level resistance to ceftriaxone ([MIC- 64 mg/ l) in *S. typhi* and *S. paratyphi* [A] [16].

If the current rate of increase in resistance to antimicrobial agents continues, it is possible that we may see the return of the pre-antibiotic era, i.e., the emergence of the post-antibiotic era [7]. Over the years the number of new antibiotics coming to the market has decreased gradually and at present only a few antibiotics are in the pipeline. Therefore it is essential to introduce multi-disciplinary interventions to reduce the inappropriate use of antibiotics to increase the lifespan of precious antibiotics. Studies have shown a relationship between

excessive use of antibiotics, antimicrobial resistance and benefits of control [17].

Strengthening of infection control, adherence to guidelines on antibiotic usage, antibiotic stewardship and good surveillance programmes are some of the interventions that will help to minimise this ever growing problem. Many countries have introduced antibiotic stewardship programmes successfully to overcome this alarming situation [18, 19]. Introduction of a national antibiotic policy with strict implementation and a well planned stewardship programme is essential to control antimicrobial resistance in our country.

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