High frequency of multi drug resistant Gram-negative rods in two neonatal intensive care units in the Philippines

JM Litzow[1,2], **CJ Gill**[3,4], **JBV Mantaring**[5], **M Fox**[3], **M Mendoza**[6], **S Mendoza**[7], **R Scobie**[8], **WC Huskins**[9], **DA Goldman**[2], and **DH Hamer**[3,4]

[1] Department of Pediatrics, Boston Medical Center, Boston University School of Medicine, Boston, MA

[2] Department of Medicine, Children's Hospital Boston, Boston, MA

[3] Center for International Health and Development, Department of International Health, Boston University School of Public Health, Boston, MA

[4] Infectious Diseases Section, Department of Medicine, Boston University School of Medicine, Boston, MA

[5] Chief of Neonatal Medicine, Philippine General Hospital, Manila, Philippines

[6] Head, Microbiological Research Laboratory, Philippine General Hospital, Manila, Philippines

[7] Jose Fabella Memorial Hospital, Department of Neonatology, Manila, Philippines

[8] Department of Microbiology, University of Leicester, Leicester, UK

[9] College of Medicine, Mayo Clinic, Rochester, MN

Abstract

Background—Though hospital-acquired infections appear to be a growing threat to newborn survival in the developing world, the epidemiology of this problem remains poorly characterized.

Methods—Over a 10 month period, we conducted prospective longitudinal surveillance for colonization and bloodstream infections with Gram-negative rods (GNRs) among all infants admitted to the two largest neonatal intensive care units (NICUs) in Manila, the Philippines, determined antibiotic sensitivities, and calculated adjusted odds ratios (OR) for factors for bacteremia using multivariate logistic regression.

Results—Among 1,831 neonates enrolled over a 10-month period, 1017 (55%) became newly colonized and 358 (19.6%) became bacteremic with a resistant GNR, most commonly *Klebsiella* species, *Enterobacter* species, *Acinetobacter* species, and *Pseudomonas aeruginosa*. The proportion of invasive isolates with antibiotic resistance was: imipenem 20%, trimethoprim-sulfamethoxazole 41%, amikacin 52%, ampicillin/sulbactam 63%, ceftazidime 67%, and tobramycin 80%. Factors significantly associated with increased risk of bacteremia were mechanical ventilation and prematurity. Additionally, colonization with a resistant GNR was an independent risk for bacteremia. (OR 1.4, 95% CI 1.0 – 1.9)

Conclusions—Colonization with a resistant GNR was an independent risk factor for sepsis. If our data are typical, the unusually high intensity of colonization pressure and disease with multidrug-resistant GNRs at these two NICUs constitutes an emerging health care crisis in the

---

Corresponding Author: Dr. Christopher J Gill, MD, MS Corresponding Author's Institution: Boston University School of Public Health.

developing world. Improved infection control methods are therefore critically needed in developing country settings.

BACKGROUND

Neonatal mortality accounts for more than one third of all global child deaths each year.(1) Sepsis is a leading cause of death within the first month of life and is often acquired through vertical transmission or unhygienic care practices in healthcare facilities.(2-6) Hospital acquired neonatal infections have emerged as a significant health problem in developing areas, (3, 4, 7-9). Nosocomial infections due to multiply resistant strains of gram negative rods appears to be a particular problem in some settings, though the extent of this remains poorly characterized.

We recently published results on an interventional cohort study that combined epidemiologic surveillance for pathogen colonization with a package of infection control interventions at two neonatal intensive care units (NICUs) in Manila, the Philippines.(10) The primary analysis revealed a high frequency of colonization and bacteremia due to gram-negative rods (GNRs).(10) This analysis was conducted to characterize the epidemiology of these pathogens at the two NICUs.

METHODS

The epidemiologic survey was conducted between May 2003 and August 2004 at two NICUs in Manila, the Philippines: Philippines General Hospital (PGH) and Dr. Jose Fabella Memorial Hospital (JFMH). Hospital characteristics and study design are described in the main results article. Gill, 2009 #5204}The study was approved by the Institutional Review Boards of Boston University School of Medicine and PGH; neonates were enrolled if mothers provided informed consent.

Laboratory methods

We performed prospective, longitudinal surveillance for colonization with gentamicin- or third generation cephalosporin-resistant GNR using stool or peri-rectal surveillance cultures collected on NICU day 0, 2, 7, weekly thereafter, and on the day of discharge among all neonates admitted to the two NICUs. The laboratory methods and collection system are described in the main effect article.(10) Blood cultures were collected from neonates with suspected sepsis according to the clinical judgment of the NICU clinicians. In contrast with the colonization surveys, our reporting of Gram-negative rods identified in blood cultures was not limited to isolates resistant to ceftazidime and/or gentamicin, but included all isolates.

All screening cultures were processed at the PGH microbiology research laboratory. Quality control procedures were established using standard resistant and susceptible American Type Culture Collection (ATCC™, George Mason University Research Laboratory, Manassas, VA) strains of target pathogens. Standard methods were used to obtain and analyze blood cultures. Positive cultures were referred to the research lab for confirmation and further characterization using BACTEC (Becton Dickinson, Franklin Lakes, NJ).

Statistical analyses

The primary outcome was incidence density (number of isolates per 100 patient-days at risk) of colonization with gentamicin- or third generation cephalosporin-resistant GNRs or GNR bacteremia. Positive cultures with isolates of identical genus and species (and susceptibility) were counted only once, though a given infant could have provided data for more than one isolate with a different genus and species. We calculated the incidence densities for

bacteremia and colonization independently of each other. To contrast incidence density between the two hospital NICUs, we calculated incidence rate ratios (IRRs) and corresponding 95% confidence intervals (CI). Patients were censored at death or discharge from the NICUs. We conducted multivariate logistic regression analyses to calculate adjusted odds ratios with 95% CI of clinical variables. Variables included in the final model were hospital, sex, birth weight, gestational age, central venous catheter use, mechanical ventilation, and whether a given infant was colonized with a gentamicin or third generation cephalosporin-resistant GNR during their NICU stay. To estimate the proportion of GNR isolates that would be treatable with a given antibiotic if used as empiric therapy, we calculated a weighted average of the proportion of GNR isolates that would be effectively treated by each antibiotic. This factored in the relative frequency of each pathogen causing sepsis within our data set, multiplied by the proportion that was sensitive to a given antibiotic. All analyses were performed with SAS 9.2 and Microsoft Excel.

RESULTS

Table 1 describes the clinical characteristics of the neonates.There were 1,831 neonates enrolled at both NICUs during the study period (Table 1). Most of the neonates were admitted with low birth weight and prematurity. At PGH and JFMH, 83% and 70% of neonates, respectively, were admitted directly from the labor and delivery ward on the calendar day of their birth. There were significant differences in the use of invasive devices between the two NICUs; significantly more newborns at JFMH had central venous catheters or were mechanically ventilated.

The cumulative incidence of neonates who were colonized or bacteremic with a resistant GNR is shown in Table 2. Surveillance cultures yielded a total of 1,997 resistant GNRs. 376 newborns became bacteremic with a total of 437 GNRs. More than half of the neonates became colonized with resistant GNRs during their NICU admission and nearly 20% had a positive blood culture. The most common GNRs identified in both colonizing and invasive isolates were *Klebsiella* species, *Acinetobacter* species, *Pseudomonas aeruginosa*, and *Enterobacter* species. In addition, a high proportion of colonization and bacteremia (76.0% and 34.3%, respectively) at the two NICUs was with non-enteric GNRs.

Table 3 summarizes the GNRs identified in blood cultures and their antibiotic susceptibility profiles. Among the most common pathogens isolated, multi-drug resistance was the rule rather than the exception. For example, the majority of *Klebsiella* spp. were resistant to ampicillin/sulbactam, ceftazidime, gentamicin and tobramycin, with most *Enterobacters* also being resistant to amikacin. Resistance rates were even higher among the common non-enteric GNRs, with >70% of *P. aeruginosa* isolates resistant to 7 of the 9 classes of antibiotics tested. In fact, the only agent with reliable activity against *P. aeruginosa* was piperacillin/tazobactam, and even here, 20% of the isolates were resistant. The most consistently active antibiotics, given the spectrum of pathogens encountered, were imipenem, piperacillin/tazobactam, and cefepime, but none of these would have reliably covered the full spectrum of pathogens causing sepsis in this population. Conversely, ampicillin/sulbactam, gentamicin, tobramycin, and trimethoprim-sulfamethoxazole showed unacceptably low rates of activity against all of the isolates tested.

To better characterize the rates of colonization and bacteremia with resistant GNRs by controlling for patient exposure time at risk, we calculated incidence densities for resistant GNRs. Because such a high proportion of isolates were non-enteric GNRs, such as *P. aeruginosa*, the analyses were stratified into enteric vs. non-enteric GNRs.

Table 4 summarizes the incidence densities and incidence rate ratios (IRR) for both NICUs. As expected, incidence densities for colonization rates were far higher than for bacteremia and particularly high at JFMH. Table 5 shows the adjusted and unadjusted odds for the occurrence of bacteremia. Not surprisingly, the factor associated with the highest risk of bacteremia was being at the JFMH NICU. Among the patient characteristics, gestational age less than 29 weeks was a significant risk factor for bacteremia. Additionally, birth weights of 1500-2000 grams, and 1000-1499 grams were both associated with increased risk for sepsis in univariate analysis. However, these risks vanished after multivariate adjustments, while the risk associated with prematurity increased further.

The incidence density of sepsis in extremely low birth weight (< 1000 grams) infants remained significantly lower compared with the higher weight category newborns (sepsis episodes per 100 patient days: 1.1 for <1000 grams, vs. 3.8 for the >2500 grams, p<0.05), despite the paradoxically associated reduced odds of invasive disease.

DISCUSSION

We identified an alarming burden of resistant GNRs at these two NICUs in the Philippines. The majority of neonates rapidly became colonized with resistant GNRs and blood culture-proven sepsis with these pathogens was highly prevalent. A high proportion of these pathogens include bacteria typically understood to be environmental organisms, suggesting that the source of these pathogens was not maternal transfer during delivery, but more likely from nosocomial spread of organisms through poor infection control. Infrequent hand-washing, inadequate equipment cleaning and exposure to other unhygienic care practices in medical facilities all aid the transfer of nosocomial pathogens. (2-6) Our study revealed that premature and low birth weight infants were at increased risk of invasive disease, particularly those requiring mechanical ventilation.

The high burden of infection and the degree of antibiotic resistance seen in our study population may signify an emerging trend among neonatal sepsis isolates from developing country NICUs. In a review of data from developing countries, Zaidi *et al* found rates of neonatal infections in hospitalized infants to be 3-20 times higher than those in developed countries, and approximately 70% of these infections would not be susceptible to treatment with conventional empiric antibiotic regimens, such as ampicillin with gentamicin.(9) There is also evidence of emerging resistance to third generation cephalosporins and quinolones. (8) A myriad of problems result: inability to adequately treat infections caused by these organisms is likely linked to the high rates of morbidity and mortality at these units, the use of broad spectrum antibiotics is expensive, these pathogens can spread to other patients, and the need to empirically use broad spectrum antibiotics can itself further contribute to an epidemic of multidrug-resistant pathogens within units.(11-15)

We were unable to explain the paradoxical inverse association between extremely low birth weight and rates of sepsis. This may reflect residual confounding, the fact that only small numbers of subjects were involved, and uncertainties regarding the direction of causality. An additional possibility is that this group is more likely to die from non-sepsis causes, such as lung prematurity or necrotizing enterocolitis. Unfortunately, absent cause of death data for the infants, we could not test this hypothesis.

In conclusion, we encountered high rates of colonization and bacteremia with resistant GNRs in two NICUs in the Philippines. As neonatal intensive care becomes more readily available in resource poor countries, improved infection control practices are an absolute necessity. Empiric antibiotic therapy for treatment of neonatal sepsis should be adjusted according to local surveillance and susceptibility data, potentially even within different

wards of a given institution. However, given the extent to which resistant pathogens have already become an important cause of neonatal sepsis, interrupting the transmission of these pathogens through improved infection control techniques must be a priority.

Acknowledgments

REFERENCES

1. Lawn JE, Cousens S, Zupan J. 4 million neonatal deaths: when? Where? Why? Lancet. 2005; 365:891–900. [PubMed: 15752534]

2. Al-Rabea AA, Burwen DR, Eldeen MA, et al. Klebsiella pneumoniae bloodstream infections in neonates in a hospital in the Kingdom of Saudi Arabia. Infect Control Hosp Epidemiol. 1998; 19:674–679. [PubMed: 9778167]

3. Bakr AF. Intravenous lines-related sepsis in newborn babies admitted to NICU in a developing country. J Trop Pediatr. 2003; 49:295–297. [PubMed: 14604163]

4. Darmstadt GL, Nawshad Uddin Ahmed AS, Saha SK, et al. Infection control practices reduce nosocomial infections and mortality in preterm infants in Bangladesh. J Perinatol. 2005; 25:331–335. [PubMed: 15716984]

5. Macias AE, Munoz JM, Galvan A, et al. Nosocomial bacteremia in neonates related to poor standards of care. Pediatr Infect Dis J. 2005; 24:713–716. [PubMed: 16094227]

6. Orrett FA, Brooks PJ, Richardson EG. Nosocomial infections in a rural regional hospital in a developing country: infection rates by site, service, cost, and infection control practices. Infect Control Hosp Epidemiol. 1998; 19:136–140. [PubMed: 9510114]

7. Newman MJ. Neonatal intensive care unit: reservoirs of nosocomial pathogens. West Afr J Med. 2002; 21:310–312. [PubMed: 12665273]

8. Vergnano S, Sharland M, Kazembe P, Mwansambo C, Heath PT. Neonatal sepsis: an international perspective. Arch Dis Child Fetal Neonatal Ed. 2005; 90:F220–224. [PubMed: 15846011]

9. Zaidi AK, Huskins WC, Thaver D, et al. Hospital-acquired neonatal infections in developing countries. Lancet. 2005; 365:1175–1188. [PubMed: 15794973]

10. Gill CJ, Mantaring JB, Macleod WB, et al. Impact of enhanced infection control at 2 neonatal intensive care units in the Philippines. Clin Infect Dis. 2009; 48:13–21. [PubMed: 19025496]

11. de Man P, Verhoeven BA, Verbrugh HA, Vos MC, van den Anker JN. An antibiotic policy to prevent emergence of resistant bacilli. Lancet. 2000; 355:973–978. [PubMed: 10768436]

12. Isaacs D. Neonatal sepsis: the antibiotic crisis. Indian Pediatr. 2005; 42:9–13. [PubMed: 15695852]

13. Aurangzeb B, Hameed A. Neonatal sepsis in hospital-born babies: bacterial isolates and antibiotic susceptibility patterns. J Coll Physicians Surg Pak. 2003; 13:629–632. [PubMed: 14700488]

14. Jeena P, Thompson E, Nchabeleng M, Sturm A. Emergence of multi-drug-resistant Acinetobacter anitratus species in neonatal and paediatric intensive care units in a developing country: concern about antimicrobial policies. Ann Trop Paediatr. 2001; 21:245–251. [PubMed: 11579864]

15. Musoke RN, Revathi G. Emergence of multidrug-resistant gram-negative organisms in a neonatal unit and the therapeutic implications. J Trop Pediatr. 2000; 46:86–91. [PubMed: 10822934]

Table 1

Clinical characteristics of neonates

Variable	PGH (N = 926)	FMH (N= 905)	Total (N = 1831)	p-value
Gestational age, weeks, mean (SD)	34.6 (3.7)	34.7 (3.5)	34.7 (3.6)	0.50
>36 weeks	365 (39.5%)	324 (35.9%)	689 (37.7%)	0.40 [*]
29-36 weeks	495 (53.5%)	527 (58.4%)	1022 (55.9%)	
<29 weeks	65 (7.0%)	52 (5.8%)	117 (6.4%)	
Birth weight, grams, mean (SD)	2085 (844.6)	2086.3 (793.2)	2085.7 (819.4)	0.97
2500 grams	319 (34.5%)	261 (28.9%)	580 (31.7%)	0.72 [*]
1500-2499 grams	341 (36.9%)	410 (45.4%)	751 (41.1%)	
1000-1499 grams	200 (21.6%)	179 (19.8%)	379 (20.7%)	
< 1000 grams	65 (7.0%)	53 (5.9%)	118 (6.5%)	
Female	399 (43.1%)	342 (37.9%)	741 (40.5%)	0.02
Any antibiotic treatment	884 (95.5%)	887 (98.0%)	1771 (96.7%)	<0.01
Central venous catheterization	254 (27.4%)	583 (64.4%)	837 (45.7%)	<.0001
Mechanical ventilation	318 (34.3%)	808 (89.3%)	1126 (61.5%)	<.0001

Figures represent N (%) unless otherwise indicated

[*] Mantel Haenzel Chi Square p-value, across the multiple strata

Table 2

Cumulative incidence of colonization and bacteremia with resistant GNR colonization or bacteremia

Colonization	PGH (N=926)		JFMH (N=905)		Total (N=1831)	
	Number	(%)	Number	(%)	Number	(%)
No. of infants who became colonized with a resistant GNR	376	40.6	641	70.8	1017	55.5
Klebsiella spp.	330	35.6	533	58.9	863	47.1
Acinetobacter spp.	42	4.5	306	33.8	348	19.0
Pseudomonas aeruginosa	62	6.7	188	20.8	250	13.7
Enterobacter spp.	79	8.5	108	11.9	187	10.2
Escherichia coli	61	6.6	77	8.5	138	7.5
Stenotrophomonas spp.	29	3.1	61	6.7	90	4.9
Alcaligines faecalis	34	3.7	51	5.6	85	4.6
Other	12	1.3	24	2.7	36	2.0
Total Resistant GNRs detected *	649		1348		1997	

Bacteremia	Number	(%)	Number	(%)	Number	(%)
No. of infants who had GNR	59	6.4	299	33.0	358	19.6
Klebsiella spp.	9	1.0	155	17.1	164	9.0
Enterobacter spp.	17	1.8	92	10.2	109	6.0
Pseudomonas aeruginosa	2	0.2	53	5.9	55	3.0
Alcaligenes faecalis	21	2.3	8	0.9	29	1.6
Escherichia coli	0	0.0	25	2.8	25	1.4
Pseudomonas other	3	0.3	17	1.9	20	1.1
Stenotrophomonas spp.	3	0.3	8	0.9	11	0.6
Acinetobacter spp.	1	0.1	7	0.8	8	0.4
Other	5	0.5	11	1.2	16	0.9
Total GNRs causing bacteremia *	61		376		437	

Abbreviations: JFMH – Jose Fabella Memorial Hospital; GNR – Gram negative rod; PGH – Philippines General Hospital

*
Totals for given pathogens was higher than the infant totals because some infants were colonized or bacteremic with more than one pathogen

Table 3

Antibiotic sensitivity profiles for invasive GNRs causing bacteremia

	Total	Ampicillin/Sulbactam	Piperacillin/Tazobactam	Imipenem	Ceftazadime	Cefipime	Gentamicin	Amikacin	Tobramycin	Trim/Sulfa
			Percentage of isolates susceptible to each antibiotic (% (n/N))							
Klebsiella spp.	164	47% (68/145)	76% (125/164)	96% (127/132)	18% (29/157)	96% (142/148)	13% (15/120)	55% (78/143)	13% (15/118)	86% (102/119)
Enterobacter spp.	109	18% (12/67)	75% (80/106)	97% (57/59)	34% (35/104)	95% (89/94)	32% (14/44)	54% (50/93)	28% (11/40)	41% (17/41)
Pseudomonas aeruginosa	55	.00% (0/6)	80% (44/55)	31% (17/55)	28% (15/54)	81% (44/54)	20% (11/54)	18% (10/55)	17% (9/54)	9% (5/55)
Alcaligines faecalis	29	25% (6/24)	100% (26/26)	96% (23/24)	96% (25/26)	15% (4/26)	50% (4/8)	25% (2/8)	38% (3/8)	100% (8/8)
Escherichia coli	25	53% (9/17)	100% (24/24)	100% (9/9)	48% (11/23)	100% (18/18)	50% (2/4)	70% (14/20)	50% (2/4)	25% (1/4)
Pseudomonas other spp.	20	0% (0/3)	100% (20/20)	75% (3/4)	35% (7/20)	100% (18/18)	50% (3/6)	61% (11/18)	100% (3/3)	67% (2/3)
Stenotrophomonas spp.	11	0% (0/3)	64% (7/11)	27% (3/11)	73% (8/11)	64% (7/11)	36% (4/11)	27% (3/11)	27% (3/11)	100% (11/11)
Acinetobacter spp.	8	100% (3/3)	13% (1/8)	38% (3/8)	13% (1/8)	13% (1/8)	25% (2/8)	14% (1/7)	25% (2/8)	25% (2/8)
		0.37	0.79	0.80	0.33	0.86	0.33	0.48	0.20	0.59

Proportion of isolates that would be sensitive to each antibiotic if used as empiric therapy

Table 4

Incidence densities and incidence rate ratios for colonization and bacteremia with resistant GNRs[*]

	N	Hospital	Person-Days	Incidence Density (events/100 person-days)	Incidence Rate Ratio (95% CI)
Time to first resistant GNR colonizer	376	PGH	5676	6.6	Reference
	641	JFMH	4149	15.4	2.3 (2.1-2.7)
Time to first non-enteric resistant GNR colonizer	137	PGH	5676	2.4	Reference
	462	JFMH	4149	11.1	4.6 (3.8–5.6)
Time to first enteric resistant GNR colonizer	304	PGH	5676	5.4	Reference
	489	JFMH	4149	11.8	2.2 (1.9-2.5)
Time to first blood culture positive with resistant GNR	59	PGH	5676	1.0	Reference
	299	JFMH	4149	7.2	6.9 (5.2-9.2)
Time to first blood culture positive with non-enteric resistant GNR	34	PGH	5676	0.6	Reference
	95	JFMH	4149	2.3	3.8 (2.6-5.7)
Time to first blood culture positive with enteric resistant GNR	27	PGH	5676	0.5	Reference
	240	JFMH	4149	5.8	12.2 (8.2–18.1)

Abbreviations: CI – Confidence interval; JFMH – Jose Fabella Memorial Hospital; GNR – Gram negative rod; PGH – Philippines General Hospital

[*] Since calculations were censored after the first event had occurred, the patient days at risk, and the total number of pathogens cannot be compared directly across the subsections.

Table 5

Risk factors for bacteremia with resistant GNRs

Variable	Exposure	Bacteremic?		Total	Odds Ratios (95% CI)	
		Yes	No		Unadjusted risk	Adjusted risk [*]
Hospital	PGH	59 (6%)	876 (94%)	926	Reference	Reference
	JFMH	299 (33%)	606 (67%)	905	4.8 (3.6-6.5)	4.1 (2.9 – 5.7)
Sex	Female	148 (20%)	593 (80%)	741	Reference	Reference
	Male	209 (19%)	878 (81%)	1087	1.0 (0.8-1.2)	0.8 (0.6 - 1.1)
Birth weight	2500+g	92 (16%)	488 (84%)	580	Reference	Reference
	1500-2499g	168 (22%)	583 (78%)	751	1.4 (1.1-1.8)	1.0 (0.7 – 1.4)
	1000-1499g	87 (23%)	292 (77%)	379	1.3 (1.0-1.7)	0.9 (0.6 – 1.5)
	<1000g	10 (9%)	108 (92%)	118	0.4 (0.2-0.7)	0.2 (0.1-0.5)
Gestational age	>36 weeks	107 (16%)	582 (85%)	689	Reference	Reference
	29-36 weeks	228 (22%)	794 (78%)	1022	1.5 (1.2-1.9)	1.5 (1.0 – 2.1)
	<29 weeks	22 (19%)	95 (81%)	117	1.0 (0.6-1.5)	2.5 (1.2 – 5.1)
Central venous catheterization	No	127 (13%)	867 (87%)	994	Reference	Reference
	Yes	231 (28%)	606 (62%)	837	2.1 (2.1-3.3)	1.1 (0.8 – 1.5)
Mechanical ventilation	No	39 (6%)	666 (95%)	702	Reference	Reference
	Yes	319 (28%)	807 (72%)	1126	6.8 (4.8-9.6)	2.8 (1.8 – 4.3)
Colonized by resistant GNR	No	103 (13%)	711 (87%)	814	Reference	Reference
	Yes	255 (25%)	762 (75%)	1017	2.3 (1.8-3.0)	1.4 (1.0 – 1.9)

Abbreviations: JFMH – Jose Fabella Memorial Hospital; GNR – Gram-negative rod; PGH – Philippines General Hospital

[*] The relative risks were adjusted for all of the other variables listed on this table.