

## **Epidemiological and bacteriological investigation of *Serratia marcescens* epidemic in a nursery and in a neonatal intensive care unit**

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### SUMMARY

An epidemic caused by *Serratia marcescens* that involved 26 infants admitted to the Neonatal Intensive Care Unit (NICU) and 82 infants admitted to the Nursery of the 2nd Medical School of Naples is reported. Two different biotypes of *S. marcescens* with two completely different epidemiological patterns were identified. The prevalent biotype (A8b trigonelline -) was isolated in the delivery room, in the operating room, in the Nursery and in the NICU from items, healthy infant excretors and affected infants; the second biotype (A3a) was isolated only in the NICU from staff, two healthy infant excretors and two affected infants. Colonization of the throat and the gastrointestinal tract was frequent. Infected and colonized infants were the most important reservoir for serratia in the Nursery and in the NICU particularly for the type strain A3a. A mucus aspiration apparatus contaminated in the delivery room and the contamination of several instruments and items probably had a major role in the initiation and maintenance of the spread of the A8b strain. Mass contamination of the nursery has been related to overcrowding and a lack of the control measures; the transfer of high-risk colonized infants caused spread in the NICU.

In the NICU the attack rate was 26%; 69% of infants became ill; the case fatality ratio was 19%. Epidemiological investigation of the infants at risk showed some factors predisposing to infection with serratia. The hygienic measures failed to control the spread of serratia and it was necessary to refuse new admissions to pregnant women in order to decontaminate and re-organize the wards.

### INTRODUCTION

There has been an increase in the isolation of serratia species from outbreaks of infection in hospitals over the last 20 years. In 1962, Ewing, Johnson & Davis reported that most serratia infections originated in hospital; later papers described the characteristics of serratia infection in the urinary tract (Taylor & Keane, 1962; Lancaster, 1962; Clayton & Von Graevenitz, 1966; Allen & Conger, 1969), in the respiratory tract (Cabrera, 1969) and at several different sites (McCracken & Lipscomb, 1965; Stenderup, Faergeman & Ingerslev, 1966; Stamm *et al.* 1976;

Mutton, Brady & Markness, 1981; Anagnostakis *et al.* 1981). Interest in such organisms stimulated several studies on their antigenic composition and on the possibilities of serological, biochemical and bacteriocin typing (Traub, Raymond & Startzman, 1971; Farmer, 1972; Edwards & Ewing, 1972; Grimont & Grimont, 1978; Branca *et al.* 1979).

In this paper are reported the results of a survey carried out during an epidemic spread of *S. marcescens* in the Neonatal Intensive Care Unit and in the Nursery of the 2nd University Hospital of Naples.

#### *General background*

The 2nd University Hospital is built on a 40 ha site and consists of 19 buildings, each forming one or more departments, connected by tunnels and passages and endowed with 1700 beds. One building contains the Paediatric Department with three Paediatric wards and one Neonatal Intensive Care Unit (NICU); in a connected building there are the Obstetrical Department and the Nursery. The NICU (optimal capacity 10 beds) and the Nursery (optimal capacity 40 beds) are virtually unified. The medical staff, working on a rota basis, is common to both wards but the nursing staff is different for each one. Maternal breast-feeding takes place in rooms annexed to each ward.

Early in November 1982 the isolation of *S. marcescens* strains from some newborns, admitted to NICU in late October, was reported to the Epidemiology Service of the Department of Hygiene; from November 4 the Service monitored daily all the newborns in the NICU. The epidemiological and bacteriological data showed a wide human and environmental spread of the organism; from November 19 the survey has been extended to the Nursery. Some special control measures were undertaken, particularly (1) negative infants and excretors were transferred to separate rooms; (2) the newly born were admitted to a separate room; (3) gowns were worn by all persons entering the rooms; (4) masks were worn by all persons responsible for the care of high risk infants in the isolation room; (5) hands were washed and disinfected on entering and leaving the rooms; (6) sterile disposable gloves were worn by all persons who were in direct contact with the newborn or with items contaminated with excreta; (7) a 4% chlorhexidine solution was used for washing hands; (8) a 1% QACs - diguanide (cetrimide 15% + chlorhexidine 1.5%) solution was used to disinfect the environment. The evident impossibility of controlling the spread by these special measures and the heavy contamination and colonization found also in the Nursery, indicated the need to refuse admission of pregnant women at term in order to empty the Nursery and the NICU for a final disinfection and reorganization. After the reopening (in February) of the wards up to June 1983 and no new cases of serratia infection were registered.

### MATERIALS AND METHODS

#### *Epidemiological survey*

A retrospective chart review of clinical and laboratory data for the period June—October 1982 has been carried out to verify the previous isolations of serratia from newborns admitted to the NICU.

*Longitudinal study.* The epidemiological and bacteriological surveillance started

on 4 November in the NICU and on 19 November in the Nursery. Each newborn with clinical symptoms and positive culture has been considered as a 'case'; the newborns with positive culture only have been considered as 'excretors'. Charts of all newborns were abstracted for date of birth, birth weight, mode of delivery, sex, symptoms, parenteral therapy, intervals and modes of feeding, and antibiotic therapy. Epidemiological analysis was carried out using appropriate subgroups of newborns. Chi-square distribution with Yates correction and Fisher's exact test were used for analysis.

### *Bacteriological controls*

#### *NICU*

Thirty one infants born since November 4 were observed; pharyngeal and rectal swabs were obtained on admission and other bacteriological tests were carried out as indicated every 3–5 days from each infant. In total 123 pharyngeal swabs, 156 rectal swabs, 41 eye fluids, 13 umbilical swabs, 60 blood cultures, 12 bronchial aspirates, 11 spinal fluids and 12 urine samples were examined.

#### *Nursery*

Between 19 November and 17 December, 115 of 151 newborns were tested by pharyngeal and rectal swabs; 81 newborns were tested at least twice within 24 h of birth and 3–7 days later.

#### *Personnel and environment*

Hands, pharyngeal and rectal swabs of the personnel (15 physicians; 40 nurses of the NICU – 30 nurses of the Nursery) were examined by hand rinse technique and swab culture.

The environmental microbiological sampling was carried out (dates: 4, 19, 30 November; 5, 15 December) by means of alginate swabs and contact plate (Rodac plate, Falcon); that sampling involved the surfaces of several structures, instruments, facilities, sanitary devices and disinfectants from the Nursery, the NICU, operating room and the delivery room making a total of 150 samples.

#### *Serratia marcescens isolation technique*

Microbiological investigation of the samples was carried out by the methods described by Sonnenwirth (1980). Pharyngeal and faecal swabs were plated in duplicate, directly and after an overnight enrichment for *Serratia* spp. in peptone water with 0.15% bile salts n.3 (Oxoid Limited, Basingstoke, Hampshire, England), on MacConkey agar (BBL, Cockeysville, Maryland, U.S.A.) containing 200000 i.u./ml colistine (UCB SpA, Turin, Italy).

#### *Identification, biochemical and serological typing*

Colonies resembling *Serratia* spp. were transferred to triple sugar iron agar (Oxoid Ltd) and lysine iron agar (Oxoid Ltd) and eventually tested for DNase production in DNase agar (Difco, Detroit, Michigan, U.S.A.) containing toluidine blue (Merck, Darmstadt, Germany) and for gelatinase production by charcoal gelatin discs (BioMérieux, Marcy – l'Etoile, France). *Serratia* strains were identified by the API 20 system for Enterobacteriaceae (API System S.A., Le Balme les

Grottes, France). The species identification was performed by utilization of adonitol, L-arabinose, D-sorbitol and L-rhamnose (Fluka; Buchs, Switzerland) as a carbon source as described by Grimont & Grimont (1978). Biochemical typing of all isolated strains was carried out by the same technique using benzoic acid, DL-carnitine, meso-erythritol, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, D-quinic acid, trigonelline (Fluka), lactose (Merck) and the production of prodigiosin and the tetrathionate reduction test. Confirmation of the biotyping and the serotyping were performed by the French National Centre at the Institut Pasteur.

#### *Antibiotic sensitivity pattern*

All strains were examined by Kirby-Bauer sensitivity testing (Bauer *et al.* 1966) in Mueller-Hinton agar (Oxoid Ltd) with commercial discs (Oxoid Ltd).

## RESULTS

### *Epidemiology and clinical illness*

#### *NICU*

The retrospective chart review did not reveal any infant positive for *S. marcescens* in the NICU during May, June and July. The first positive case was recorded in August from a preterm infant with severe birth defects transferred to the ward from another hospital. Throughout the period from August to October 77 infants were discharged from the NICU: in the same period seven 'cases' (9%) could be identified. The serratia isolations were distributed as follows: two in August, one in September and four in October. Two cases admitted in late October were discharged in November and another died in December. The graph of admissions, discharges and of the serratia isolations during November and December is shown in Fig. 1.

Since our survey started we have surveyed daily eight newborns (n.) previously admitted in October (n. 1-8) and 23 (n. 9-31) admitted between 4 November and 15 December. Initially, 3 of 8 (38%) newborns were colonized; subsequently 19 of 23 (83%) newborns became colonized. The daily positivity rate varied from 14 to 58% until 15 December and, after a temporary reduction, reached the 100% level because of cross-contamination of all the remaining five infants; this did not alter until their progressive discharge.

The periodic bacteriological controls showed that during November and December 10 of 23 (44%) infants acquired the infection during birth or in the Nursery before the transfer to the NICU and 9 of 23 (39%) acquired it 3-24 days after admission to the NICU; only 4 of 23 (17%) were negative in all the tests. In only one case (n. 11) did an infant become free of infection spontaneously within 8 days. Antibiotic treatment of affected infants did not modify the pharyngeal or the intestinal carriage of the organism.

The epidemic curve of the outbreak is presented in Fig. 2. Throughout August-December 26 of 100 infants admitted to the NICU became infected. The first two cases to occur may have been connected; later only two sporadic cases were identified. The increase in isolations reached maximum values in the second and fourth week of November. During the first three weeks of December the decrease of admissions resulted in a slight reduction of the spread followed by an

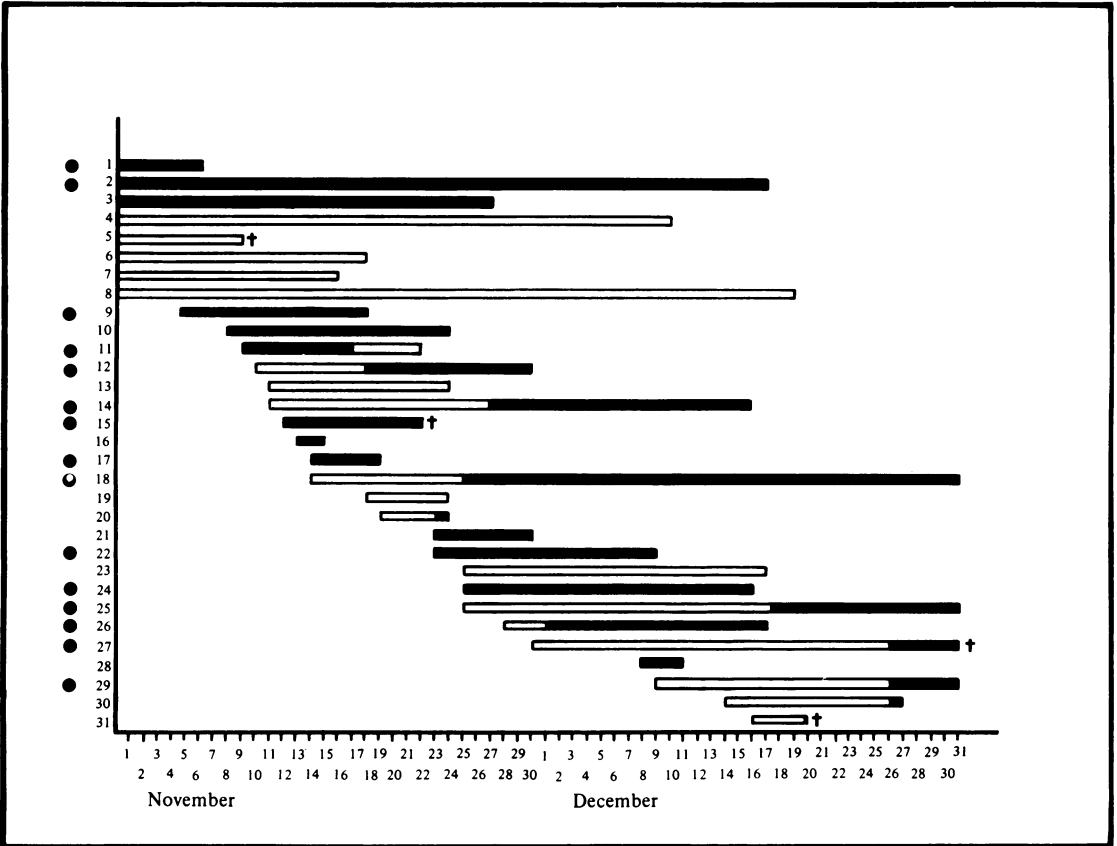


Fig. 1: Schema of admissions, discharges and serratia isolations in the Neonatal Intensive Care Unit during November and December. ●, Newborns with clinical illnesses. †, Dead. ■, Positive for *S. marcescens*. □, Negative for *S. marcescens*.

unexplained incident which caused the cross-contamination of the last newborns. During these last months the incidence rates were respectively 81 and 54%. Statistical analysis did not show significant differences in the type of care among infected and non-infected patients.

The clinical illnesses related to serratia infections are presented in Table 1; 18 of 26 newborns (69%) showed one or more clinical signs of infection. Although several manifestations of disease were observed, the prevalent ones were bronco-pneumonia, diarrhoea and sepsis, the first two involving over 50% of the affected infants; three conjunctival infections were observed and three cases (A2 - 25 - 27) developed respectively, elbow abscess and purulent arthritis of the knee joint; 8 of 26 symptomless newborns (31%) were considered healthy excretors. The overall case fatality ratio was 5 of 26 (19%); it was lower during November and December, but was particularly high among the cases of bronchopneumonia occurring in infants admitted with cardiac or pulmonary birth defects. Clinical illness caused by *S. marcescens* has always complicated underlying disease processes and this

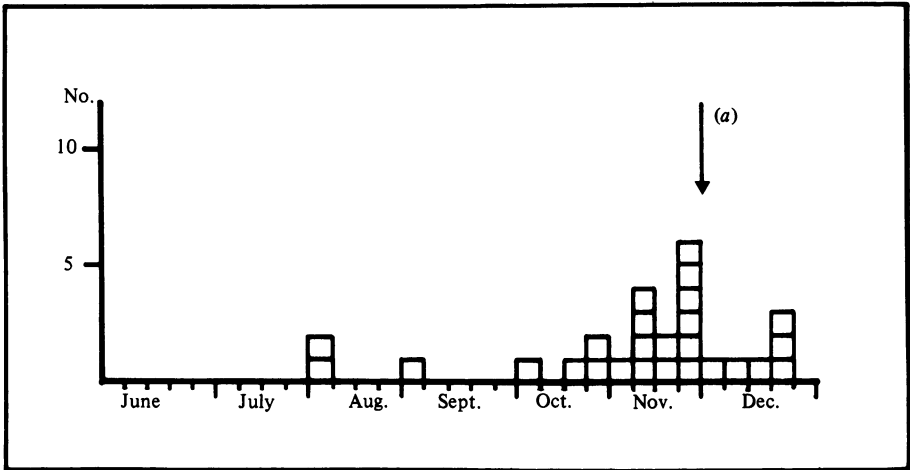


Fig. 2: Epidemic curve of the serratia outbreak in the Neonatal Intensive Care Unit (NICU). (a) Closure to new admissions of at term pregnant women.

Table 1. *Clinical illnesses in newborns of NICU infected with serratia*

Clinical illness	Patient (Case no.)																		
	A1	A2	S3	1	2	9	11	12	14	15	17	18	22	24	25	26	27	29	
	†		†		†					†								†	
Broncopneumonia	+		+		+				+	+		+							
Diarrhoea						+	+				+		+			+			
Sepsis		+				+				+	*					+	*	+	+
Joint abscess															+				
Purulent arthritis																		+	
Urinary infection				+															+
Meningitis		+																	
Conjunctivitis									+						+				+

In addition the following patients were asymptomatic excretors: 0c4; 3; 10; 16; 20; 21; 28; 30.

\* With isolation of *S. marcescens* from blood.

† Dead.

relationship is evident from the analysis of differences in weight and gestational age of affected (mean 2260 g) and non-affected infants (mean 2860 g). The pre-term infants were exposed to a relative risk (r.r.) of developing clinical illness from serratia infection twice that of full term infants (r.r. = 2.1); this value grows dramatically if computed on all newborns in the same period admitted to the Nursery and becoming excretors (r.r. = 19.4).

The prophylactic administration of ampicillin and gentamicin was started from birth in 12 of 26 newborns but did not avoid the subsequent colonization or the onset of the clinical illness (chi-square = 1.86; Yates correction).

Table 2. Daily percentage of positive samples among pharyngeal and rectal swabs from infants of Nursery

Date	Number examined	Number (percentage) positive
November		
19	19	14 (73·7)
20	17	10 (58·8)
21	21	9 (42·9)
22	21	10 (47·6)
23	30	16 (53·3)
24	28	17 (60·7)
25	31	14 (45·2)
26	28	10 (35·7)
27	34	16 (47·1)
28	22	12 (54·5)
29	30	20 (66·6)
30 (a)	32	22 (68·8)
December		
1-16	54	39 (72·2)
Total	367	209 (56·9)

(a) Closure to new admissions.

*Nursery*

The survey was extended to the Nursery when the early data indicated that 50% of the newborns were becoming infected before their admission to the NICU. The findings showed a large spread of *S. marcescens* and also a very high percentage of infected infants in the Nursery. Throughout the early period of the survey (19-30 November) the daily frequency of positive infants ranged between 36 and 74% with an average of 54%; in the weeks after closure to admissions, with the subsequent progressive decrease in the number of newborns and a slight increase of the observation time of the positive newborns, the frequency average has risen to 72% (Table 2). The newborns were examined within 24 h and 3-7 days after birth in order to determine the rapidity and the type of colonization; 40% of the newborns became positive within 24 h of birth and 71% within 7 days; the value reached 84% for those newborns examined after 8 days (Table 3). The percentage of infected infants decreased after 24 November following the disinfection of one mucus aspirator found contaminated with serratia. Over half of the newborns were found to be both pharyngeal and faecal excretors.

In only 6 of 82 (7%) of positive newborns from Nursery did a mild form of diarrhoea appear significantly related to the serratia infection.

*Personnel and environmental controls*

Bacteriological observations on the personnel gave rather surprising results; positive samples were found only in the personnel working in NICU, and particularly among the 40 nurses; three (7·5%) were pharyngeal carriers, one (2·5%) was a faecal carrier and three (7·5%) hand carriers. All appeared healthy and were most probably infected during direct contact with infants or as result of handling contaminated items.

Table 3. Results of the pharyngeal and rectal swabs examined within 24 h and 3-7 days after birth

Birthdate	Total	Examined 24 h			3-7 days			Not examined*
		Number	Pos.	%	Number	Pos.	%	
Before Nov. 16	37	—	—	—	18	13	72.2	19
November								
16-18	20	—	—	—	16	6	37.5	4
19	11	9	3	33.3	9	5	55.5	2
20	6	4	2	50.0	4	3	75.0	2
21	12	11	7	63.6	11	7	63.6	1
22	5	4	2	50.0	4	2	50.0	1
23	2	0	0	—	0	0	—	2
24	8	8	0	0	8	4	50.0	0
25	7	6	3	50.0	6	5	83.3	1
26	6	6	2	33.3	6	6	100.0	0
27	7	6	2	33.3	6	6	100.0	1
28	7	7	2	28.6	7	7	100.0	0
29	8	8	3	37.5	8	7	88.9	0
November 30 to	15	12	5	41.7	12	11	90.9	3
December 13								
Total	151	81	31	38.3	115	82	71.3	36

\* i.e. transferred to NICU or discharged without bacteriological findings.

*S. marcescens* was found to be quite spread in the environment. Of the samples 14% (21 of 150) taken in the delivery room, operating room, nursery and NICU gave positive results. The organism was found most frequently on the surfaces of items, particularly on balances and swaddling tables (4 of 16: 25%), on cushions and mattresses (4 of 20: 20%), gauzes and incubators (4 of 42: 9.5%), on various surfaces such as floors and facilities (5 of 40: 12.5%) but never in respirators, disinfectants, humidifiers, neonatal isolettes and air (0 of 16). The Nursery environment was found to be more heavily contaminated than other wards; sampling after a first disinfection gave positive results in two handwashing basins but negative results were obtained after a further final disinfection.

#### Biochemical typing

The study of 352 biochemical patterns allowed us to divide the isolates into two different biotypes: the type A3a, already classified by Grimont & Grimont (1978), and another, A8b (trigonelline—), which shows a biochemical pattern recently recognized. This strain has several differences and in particular it utilizes, as a unique carbon source, D-quinic acid, 4-hydroxybenzoate and 3-hydroxybenzoate, furthermore it has failed to grow on media containing *m*-erythritol, trigonelline, benzoate, DL-carnitine and lactose (Table 4). Serotyping has confirmed the results of biochemical typing; biotype A3a was serotype 012:H 4687 (the H antigen is a new one previously referred to as H 4687) and biotype A8b (trigonelline—) was serotype Co14:H 12.

The two biotypes have probably had a simultaneous but different circulation; the type A3a, less prevalent, was detected only in the NICU in four infants and in the personnel but never in the environment. On the other hand the type A8b



Table 4. *Biochemical patterns of the two different S. marcescens strains identified*

	Biotype	
	A8b (trigonaelline-)	A3a
Growth on:		
<i>m</i> -Erythritol	-	+
Trigonaelline	-	-
Quinate	+	-
4-Hydroxybenzoate	+	-
3-Hydroxybenzoate	+	+
Benzoate	-	-
DL-Carnitine	-	-
Lactose	-	-
Tetrathionate reduction	+	+
Red pigment	-	-
Serotype	Co14:H12	O12:H4687

has been isolated from the Nursery, NICU, operating room and delivery room; it has only been isolated from environmental samples and was never detected in personnel. This strain has been responsible for the greater part of the infections and clinical illnesses as the A3a strains were isolated in only one case of bronchopneumonia and in one case of conjunctivitis. The strains responsible for the first four infections were not stored for typing. Strains of identical biotypes have been isolated in different sites of the same newborn but never were strains of different biotypes detected in the same infant.

#### *Antibiotic sensitivity pattern*

The sensitivity patterns of 352 *S. marcescens* strains were determined (Table 5). Although our isolates showed a pattern quite typical of the genus, sensitive to aminoglycosides, to acylureido penicillins and to cefotaxime, it may be useful to describe some interesting variations observed only in strains isolated from two newborns during antibiotic treatment.

*Case n. 25*: 16 samples; the antibiotic sensitivity pattern of the first nine isolates (four faecal, two pharyngeal, two blood, one purulent fluid) was typical of the majority of strains; the last seven isolates (six faecal, one pharyngeal) have shown the development of resistance to carbenicillin, acylureido penicillins, cefotaxime, nalidixic acid, pipemidic acid, oxolinic acid, trimethoprim-sulphamethoxazole and chloramphenicol (treatment received before the last seven isolations: cefotaxime and amikacin and six days later chloramphenicol in addition).

*Case n. 18*: the first strains isolated were resistant to carbenicillin, acylureido penicillins and cefotaxime; some days after the interruption of the treatment the samples from the newborns have shown the same pattern as the majority of *serratia* strains previously reported.

Significant differences in sensitivity pattern between the A3a and the A8b biotype strains have not been found. But in two cases a variation among biotype A3a isolates was demonstrated. The strains recovered from the newborns n. 2 and n. 12 showed the same pattern characterized by resistance to acylureido penicillins;

Table 5. *Antibiotic sensitivity patterns of 352 S. marcescens strains isolated during the epidemic*

Antibiotic	Resistant		Intermediate		Susceptible	
	Number	%	Number	%	Number	%
P, AML, PN, F, PB, KF, MA, TE	352	100	0	—	0	—
PY	13	4	339	96	0	—
MEC, PRL	13	4	0	—	339	96
CTX	9	3	0	—	343	97
K, CN, SIS, TOB, NET	11	3	0	—	341	97
AK	6	2	0	—	346	98
NA, PIP, OA, SXT, C	7	2	0	—	345	98
FOS	0	—	0	—	352	100

P, penicillin G; AML, amoxycillin; PN, ampicillin; F, nitrofurantoin; PB, polymyxin B; KF, cephalothin; MA, cefamandole; TE, tetracycline; PY, carbenicillin; MEC, mezlocillin; PRL, piperacillin; CTX, cefotaxime; K, kanamycin; CN, gentamicin; SIS, sisomicin; TOB, tobramycin; NET, netilmicin; AK, amikacin; NA, nalidixic acid; PIP, pipemidic acid; OA, oxolinic acid; SXT, trimethoprim-sulphamethoxazole; C, chloramphenicol; FOS, fosfomicin.

after some days the isolates from newborn n. 2 (treated with ampicillin and gentamicin) also showed a characteristic resistance to aminoglycosides except amikacin; strains with identical pattern were later isolated from the hands of two personnel.

#### DISCUSSION

Several *S. marcescens* outbreaks in neonatal pathology units and in nurseries have been described previously.

This outbreak, although similar to others in Italy and abroad, seems to represent one of the more extensive and complex, particularly from the epidemiological point of view, since two biotypes of *S. marcescens* were identified at the same time.

The first observation concerns the introduction of the A3a strain in the NICU which probably occurred after the transfer of an infected premature infant from another hospital; interhospital spread was also reported by Schaberg *et al.* (1976) but in that outbreak carriage on the hands of personnel was apparently implicated as the mode of interhospital spread. Stamm *et al.* (1976), Schaberg *et al.* (1976) and Jones *et al.* (1978) have reported outbreaks due to an apparent transmission between colonized patients without an environmental source sufficient to explain the epidemic; in this type of spread long-stay patients and the transmission via the hands of personnel appeared to assume a major role. This epidemiological pattern, displayed by the A3a strain, is suggested by certain observations: (a) the strain was isolated only in the NICU and only from infants and nurses of this ward; (b) hand carriage amongst the nurses was demonstrated; (c) identification and isolation measures were effective in control of the spread of this biotype.

The second strain (A8b) was introduced into the Nursery by an unknown source and, after a more extensive colonization of infants and environment, was spread from these reservoirs within the neonatal wards by equipment and high risk infants transferred from the Nursery to the NICU. This different epidemiological pattern

has been observed in several epidemics (Anagnostakis *et al.* 1981; Cleary, MacIntyre & Castro, 1981; Krieger *et al.* 1980; Mc Cormack & Kunin, 1966; Rabinowitz & Schiffrin, 1953; Rutala *et al.* 1981; Sanders *et al.* 1970) but never to a similar extent. The failure of all the control measures, which led to closure to new admissions, was undoubtedly due to heavy contamination of the environment and of several items which had a critical role in the continuous maintenance of the colonization. Amongst these items one of the most important was the mucus suction apparatus in the delivery room. The importance of the human reservoir appears evident in our epidemic as in almost all other outbreaks; like Stamm *et al.* (1976) we could not determine the duration of colonization, however the periodical tests on the infants admitted to the NICU, together with biotyping and sensitivity patterns showed the persistence of pharyngeal or gut colonization for up to 60 days. However recolonization indistinguishable on the basis of the biochemical typing can not be excluded. Bacteriocin typing might clarify this point (Branca *et al.* 1979; Farmer, 1972; Traub *et al.* 1971). Differences in duration between pharyngeal and gut carriage were not observed.

Among the factors which could have contributed to the widespread infection in the Nursery was the overcrowding observed in October (a 30% excess of newborns) with a consequent understaffing (Haley & Bregman, 1982), but this would not explain the same trend which occurred after the progressive discharge of the infants.

The study of antibiotic sensitivity patterns showed that our strains were similar to most isolates in other epidemics (Meers, Foster & Churcher, 1978; Stamm *et al.* 1976) and confirmed that multiple resistance may be acquired during treatment (Schaberg *et al.* 1976).

In general the data confirm that the epidemiological patterns of the *S. marcescens* can be as various and complex as other 'opportunists' and are closely related: (1) to the biological characters of the strain and its adaption and interaction possibilities, (2) to the environmental features, (3) to the host sensitivity, (4) to the staff organization and behaviour. Many observations remain unexplained and research and exchange of information can lead to a more effective and speedy improvement in the knowledge of hospital infections on a interdisciplinary and international basis.

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