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SERRATIA MARCESCENS : BIOCHEMICAL CHARACTERISTICS, ANTIMICROBIAL SENSITIVITY AND CLINICAL SIGNIFICANCE

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Summary

Serratia marcescens has become an important nosocomial pathogen. During a period of seven years 123 strains, both pigmented and non-pigmented varieties were isolated. Their biochemical characteristics, antibiotic susceptibility pattern and clinical significance are described. The aminoglycosides appeared to be the most effective agents against this organism. "Compromised" patients were most vulnerable to infection. Because of its potential pathogenicity, yreater awareness of the organism by both clinicians and microbiologists is necessary in the prevention and treatment of infections caused by *S. marcescens.*

INTRODUCTION

Serratia marcescens is a gram negative, motile, non-lactose fermenting bacillus of the family Enterobacteriaceae. Both pigmented and non-pigmented varieties exist. It can be found in soil and water and was originally considered to be only a saprophyte. It is now recognised as a formidable nosocomial pathogen[1] capable of causing serious infections and often death in hospitalized patients.

The organism usually attacks compromised hosts: patients with defective immune responses or some underlying severe disability to predispose them to infection or provide an artificial portal of entry. Neonates, the immunosuppressed, patients with carcinomatosis, leukaemia or a reticulosis and those with chronic neurological and urological disorders are at risk.[2] Prior corticosteriod therapy, the post-operative status, mechanical respiratory manipulation, instrumentation of the genito-urinary tract and multiple and "broad-spectrum" antibiotic therapy may also predispose to serratia infections.[3]

It has been implicated in septicaemias,[3] osteomyelitis,[4] urinary tract infections, wound infections, peritonitis as well as other types of infection.'

Treatment of these infections may be a difficult problem because many recently isolated strains have shown a high level of resistance to several antimicrobial agents. In one report[S] over half the strains tested were found to be resistant to ten or more antimicrobial agents.

Besides this, these infections usually occur in patients, in whom any infection may be difficult to control. Hence this organism is being regarded by concerned microbiologists as the "pseudomonas" of the future. The gram negative rods such as klebsiella, serratia and pseudomonas are being implicated more and more in hospital acquired infections.

The following is a report on the biochemical characteristics, antimicrobial susceptibility pattern and clinical significance of strains of *S. marcescens* isolated from clinical material. This study which extended over a period of many years from November 1973 to October 1979, was carried out at the University Hospital, Kuala Lumpur, Malaysia. This is a 800-bed teaching hospital with wards for all the major specialities.

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MATERIALS AND METHODS

Clinical specimens were cultured routinely onto blood agar and MacConkey agar plates. Chocolate agar plates were also used for blood cultures, vaginal swabs as well as ear swabs. Urine viable counts were performed on blood and MacConkey agar plates using the urostrip method of Leigh and Williams.[6] Cultures were incubated for 24–48 hours at 37°C. Identification of all isolates was based on an extensive battery of biochemical tests according to the methods described by Cowan and Steel.'

Strains isolated were tested against 19 chemotherapeutic agents by the disc-agar diffusion method using Oxoid Diagnostic sensitivity agar. Disc potencies of the drugs used (in micrograms, except for penicillin which was in units) were as follows: amikacin (30), ampicillin (25), carbenicillin (100), cefuroxime (30), cephaloridine (25), chloramphenicol (25), cotrimoxazole (25), erythromycin (5), gentamicin (10), kanamycin (30), nalidixic acid (30), neomycin (30), nitrofurantoin (200), penicillin G (1), sisomicin (10), streptomycin (10), sulphadiazine (200), tetracycline (25) and tobramycin (10).

Patients' case records were viewed for information such as age, sex, source of specimen, presence of other pathogens in mixed cultures, antimicrobial therapy as well as other relevant clinical data.

RESULTS AND COMMENTS

Bacteriology:

Classically, ***S. marcescens*** has been recognised in the laboratory by its ability to produce a characteristic red pigment. Of the 123 strains studied, 84 (68.3%) were pigmented and 39 (31.7%) strains were non-pigment producers. Edwards and Ewing[8] found that only 20.9% of their strains were pigmented. Our high (68.3%) isolation rate of the pigment producers may or may not be a true incidence for it is possible that many non-pigmented strains may not have been identified.

The biochemical reactions of the 123 isolates are given in Table 1. Most of the strains were consistent in their reactions and compared closely with those of Cowan and steel[7] as well as Eowards and Ewing,[8] with the following exceptions: gas production occurred in 15% of our strains as compared to 52.6% of Edwards and Ewing; acid production from arabinose and rhamnose occurred in 2% of our strains (negative in Edwards and Ewing).

Antibiotic susceptibility

Table 2 gives the results of disc sensitivity testing for 100 serratia strains. All strains tested were resistant to penicillin G. Ninety-eight percent of the strains were resistant to erythromycin and cephaloridine. Between 80–93% of the strains exhibited resistance to ampicillin, tetracycline, sulphadiazine and nitrofurantoin. Most strains were susceptible (0–10% resistance) to sisomicin, tobramycin, amikacin, nalidixic acid, gentamicin, neomycin, chloramphenicol and carbenicillin.

All the 100 strains both pigmented and nonpigmented were resistant to five or more antimicrobial~. Thirty-five percent of the pip mented and 42% of the nonpigmented (average 40%) strains were found to be resistant to ten or more chemotherapeutic agents.

Clinical data

The clinical sources of the 123 isolates are shown in Table 3. The organisms were isolated mainly from wound swabs (33%) especially from surgical wounds and sites of drainage tubes. Significant numbers were also isolated from the respiratory tract (15%) and the gastrointestinal tract (15%). Eleven percent of the isolates were from urine and 10% from blood. There was only one isolate from the cerebrospinal fluid.

The miscellaneous group consisted of strains from liver, breast and retroperitoneal abscesses, discharging sinus and fistula and positive cultures from the vagina, ear, ureter and umbilicus.

Sixty-four cases were analysed out of which only 60 patients were thought to have true infections due to S. ***marcescens.*** These isolates were classified as clinically significant because there was clinical evidence of infection at the time of isolation such as inflammation, a rise in temperature and increased peripheral white blood cell count; the organisms were either recovered in pure culture or recovered repeatedly as the predominant organism in mixed cultures; and the patient's favourable response to suitable antimicrobial therapy.

TABLE 1

BIOCHEMICAL REACTIONS of 123 STRAINS OF S *MARCESCENS* 84 PIGMENTED AND 39 NON-PIGMENTED

Test	Per cent positive		
	Pigmented	non-pigmented	Total
oxidase	0	0	0
motility	100	100	100
gas from glucose	14	15	15
Acid from glucose	100	100	100
lactose	10	3	7
sucrose	99	97	98
mannitol	100	100	100
arabinose	2	0	2
rhamnose	4	0	2
raffinose	7	0	5
indole (Kovacs)	3	0	2
methyl red	10	15	11
Voges-Proskauer	99	100	9
citrate (Koser's)	77	62	72
malonate	5	0	3
urease (Christensen's)	3	18	7
gelatin liquefaction	100	97	99
phenylalanine deaminase	0	0	0
lysine decarboxylase	99	97	98
ornithine decarboxylase	99	97	98
arginine dihydrolase	0	0	0
deoxyribonuclease	100	97	98
hydrogen sulphide (Kligler's)	0	0	0

The age distribution of the patients by decade showed a peak in the fifth decade – 14 patients; there were 10 in the third decade and eight each in the fourth and seventh decades. There were four infants who were less than one year of age. There were 38 males and 22 females. The ethnic distribution consisted of 37 Chinese, 12 Malays, four Indians and seven designated as other races.

S. marcescens was isolated as a pure growth from 31 cases and in 19 cases together with ***Pseudomonas aeruginosa.*** Klebsiella species and E. *coli* were other common organisms isolated in mixed cultures. There was one patient where serratia was present together with ***Pseudomonas aeruginosa*** and klebsiella species. This was a case of IgA deficiency with otitis media.

Fifty-five of the 60 clinically significant cases were either in the post-operative stage, or post-instrumentation or were infected traumatic wounds. Hence certain predisposing factors become prominent: surgery in 35 cases especially abdominal surgery (15 cases) and instrumentation in eight cases especially catheterization (6 cases). Table 4 gives the main features associated with the recovery of S. ***marcescens.***

Duration of stay in hospital appears to be an important factor in the pathogenesis of these infections. Fifty patients were hospitalized before ***S. marcescens*** was isolated. The length of hospitalization varied from one day to 71 days with an average of 21 days. Thirty-five cases were treated with antibiotics before the isolation of S ***marcescens***

TABLE 2

ANTIBIOTIC SUSCEPTIBILITIES OF 100 STRAINS OF *S. MARCESCENS:* (69 PIGMENTED AND 31 NON-PIGMENTED)

Test	% of strains RESISTANT		
	Pigmented	Non-pigmented	Total
Penicillin	100	100	100
Erythromycin	99	97	98
Cephaloridine	97	100	98
Sulphadiazine	93	94	93
Tetracycline	90	94	91
Ampicillin	93	81	89
Nitrofurantoin	87	74	83
Cefuroxime	85	55	76
Streptomycin	65	65	65
Kanamycin	29	26	28
Cotrimoxazole	16	10	13
Chloramphenicol	7	19	11
Carbenicillin	6	19	10
Gentamicin	10	3	8
Neomycin	8	0	6
Sisomicin	3	0	2
Nalidixic acid	1	0	1
Amikacin	0	0	0
Tobramycin	0	0	0

DISCUSSION

This study had two objectives, the first being to determine the antibiotic sensitivity pattern of the *S.* marcescens isolated in this hospital and compare it with published data from elsewhere and the second objective being a retrospective assessment of the clinical significance of the serratia strains which were isolated in this laboratory during the study.

It has been established beyond doubt by the large number of publications in recent years, that *S.* marcescens can behave as a pathogenic organism,[1–5] hence the importance of studying the antibiotic sensitivity pattern. Clinical isolates of *S marcescens* are usually resistant to many groups of antibiotics.

Our results of a high percentage of resistance of S marcescens to ampicillin, cephaloridine, erythromycin, penicillin and sulphadiazine correspond to previous reports concerning the inefficacy of these drugs.[9–11] Even the new cephalosporins have not shown great promise against *S.* marcescens as illustrated by our finding of 76% resistance to cefuroxime. Cooksey et *al*[10] reported that 39% of their strains were resistant to carbenicillin. This is very high compared to ours which showed 6% for the pigmented variety and 19% for the non-pigmented strains, the total being only 10%. However, this may be due to the use of different content discs in the diffusion test.

Tne aminoglycosides, kanamycin and gentamicin have been the drugs of choice for many years because of in *vitro* sensitivity and therapeutic effectiveness. However, gentamicin resistance rates of 20 to 50% have been reported.[1*2] Amikacin shows potential against *S.* marcescens but already warnings of resistant strains have appeared.'? Any further increase in aminoglycoside resistance will seriously jeopardise the effective treatment of serratia infections. Fortunately, our strains have been found to be mostly sensitive to all the aminoylycosides tested.

TABLE 3
CLINICAL SOURCES OF S. *MARCESCENS*

Source	Number of strains		
	Pigmented	Non-pigmented	Total
Respiratory tract:			
throat swab	1	1	2
tracheal secretions	2	2	4
sputum	2	1	3
pleural fluid/drain	8	2	10
Alimentary tract:			
nasogastric aspirate	1	0	1
bile	4	2	6
peritoneal fluid/drain	9	1	10
rectal swab	1	1	2
Wound swabs:			
drainage tube site	11	2	13
burns	0	1	1
skin grafts	1	1	2
surgical wounds	15	9	24
Blood	7	5	12
Urine	11	3	14
C.S.F.	1	0	1
Miscellaneous	10	8	18
Total	84 (68%)	39 (32%)	123 (100%)

In vitro synergy between trimethoprim and sulphamethoxazole against ***S. marcescens*** has been reported[9] and only **13%** of our strains were found to be resistant to cotrimoxazole. But there is a paucity of reports on serious infections due to ***S. marcescens*** being successfully treated with cotrimoxazole.

For urinary tract infections, nalidixic acid would perhaps be the drug of choice as only one percent of our strains were resistant to this drug. Other reports[10,14] are in agreement with this and nalidixic acid can be a good choice for those infections with serratia confined to the urinary tract without systemic involvement.

Conflicting results have been reported concerning antibiotic sensitivities of pigmented and non-pigmented strains.[5,16] We found no marked difference between pigmented and non-pigmented strains in their antibiotic sensitivities except for carbenicillin (6%, **19%**) and chloramphenicol (**6%. 19%**). However, more of the non-pigmented strains were multiple resistant to ten or more antibiotics.

The therapy of serious infections caused by ***S. marcescens*** will normally depend on the sensitivity pattern of the organism involved but the current drugs of choice seem to be still gentamicin and amikacin. However, with the increasing problem of resistance, it is important to study effective combinations of drugs as well as the ***in vivo*** usefulness of hitherto untried antimicrobials like sisomicin against ***S. marcescens.***

Serratia isolates are being identified with increasing frequency from hospitalized patients. There are three factors which contribute to the increased identification of this bacillus in clinical specimens.[7] First the organism has some innate drug resistance, and it easily acquires additional resistance. Second, there appears to

TABLE 4
FEATURES ASSOCIATED WITH THE RECOVERY OF *S. MARCESCENS*

Feature		No. of cases
Suryery:		35
gastrointestinal	15	
Uro-genital	6	
Renal	5	
Cardiovascular	4	
CNS	1	
Others	4	
Instrumentation:		18
Catheterization	6	
Uthers	12	
Trauma (non surgical)		2
Miscellaneous:		5
IgA deficiency	1	
Bronchiectasis	1	
Chronic renal failure	1	
Salpingitis	1	
Umbilical infection (neonate)	1	
Total		60

be a relative increase of serratia in the hospital environment. Third, clinical laboratories are able to identify the crganism more easily than before.

Additionally, increasing manipulative procedures are undertaken these days in hospitals and therefore the number of opportunistic infections would also tend to increase. We analysed 60 cases of serratia infections and 55 of these could be considered as hospital acquired infections.

O·ıt of our total of 123 isolates thirty-three per cent were from wound swabs – mainly post-operative surgical wounds and wounds associated with the site where drainage tubes were placed. Drainage tubes of the pleura and peritonium were also infected with serratia quite commonly (see Table 3).

Patients with urinary isolates formed the next big group of 11% and all of these had either been catheterized or had an indwelling catheter or both. These figures compare well with those of other reports.[4]

Twelve of our isolates of serratia were from blood (10%). Their clinical significance will however, form the subject of another paper under preparation. Serratia septicaemia is a life threatening infection that needs aggressive treatment.

There was only one isolate from cerebrospinal fluid. This was from a 62 year old female who had a craniotomy and a ventricular drain inserted for an acoustic neuroma. Serratia was cultured from the CSF two days later and tnere was some clinical evidence of meningitis. This patient died on the tenth post-operative day despite treatment with cotrimoxazole and chloramphenicol.

A very important predisposing factor in the isolation of serratia from hospitalized patients is previous antibiotic therapy. Thirty-five of the 60 cases analysed in this study received antibiotics for reasons such as post-operative treatment or prophylaxis before serratia was isolated. One to six different types of antibiotics were given per patient, the common ones being

ampicillin, tetracycline, gentamicin and combination of penicillin and streptomycin. Except for gentamicin, serratia are mostly resistant to these antibiotics and their selective pressure must have contributed to the emergence of multiple resistant strains of the organism.

It is reasonable to conclude that *S. marcescens* can be a virulent pathogen. It is present in the environment of hospitals and is an opportunistic organism preying on patients who are "compromised". All necessary precautions must be taken to prevent the occurrence and spread of this organism in hospitals. Vigilance and surveillance of nosocomial infections in hospitals should become a routine procedure. Intensive treatment of patients who have acquired such infections should be carried out with proper antimicrobial agents. Lastly, laboratory staff must be able to isolate and identify this organism rapidly and clinicians should not continue to ignore or dismiss it as a harmless contaminant.

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