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Pseudomonas aeruginosa and Proteus Infections in Premature Babies and in Infants

Therapeutic Trial with Carbenicillin

By

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The results of 705 bacteriological investigations carried out between January 24th and March 31st, 1969, in an infant and premature baby ward are reported. From the material pseudomonas strains were cultivated in 58 cases, and proteus strains in 48 cases.

The symptoms of 12 babies suffering from pseudomonas or proteus infection is discussed. In the majority of cases, pseudomonas caused mastoiditis and proteus caused pneumonia. Mastoiditis elicited by pseudomonas was not cured when treated either with the generally accepted drugs or with carbenicillin. After antrotomy, all patients but one were cured. Prophylactic carbenicillin treatment of high risk neonates seemed to be favourable.

Hospital infection of premature babies and neonates by Gram-negative, mostly facultative pathogens is an increasingly important worldwide problem. In the last few years, attention has turned towards infections caused by *Pseudomonas aeruginosa* and *Proteus* [1, 7, 8]. Our own attention has first been awakened [3] by the otitis caused by pseudomonas and proteus in premature babies.

The increase in pseudomonas infections has first been observed in hospital patients [6, 7, 11], especially in neonates and in old individuals. Premature babies were particularly frequently infected [1, 5, 11]. According to data in the literature [11], the causes are as follows.

(i) Hospital furnishings (wash basins, bath, mechanical appliances, etc.) are difficult to disinfect;

(ii) pseudomonas is resistant to the majority of (wide spectrum) antibiotics;

(iii) during treatment with antibiotics the pseudomonas strains often become more virulent;

(iv) the hospital staff often carries pseudomonas and proteus strains (in the nose, throat);

(v) weakened state, premature birth, serious basic disease, steroid or immunosuppressive treatment increase susceptibility.

Even from the epidemiological point of view, it is difficult to fight pseudomonas, since it is relatively insensitive against many disinfec-

tants and the sources of infection are also obscure and changeable. According to recent investigations, an ever increasing number of healthy persons carries pseudomonas [2]. In hospitals, infected wounds, burns, chronic pyelonephritics and those suffering from primary or secondary respiratory disease, as well as organisms hidden in fittings (water taps, furnishings) may be sources of infection. In premature baby and neonatal wards, respirators, appliances used for resuscitation, rubber tubes, etc., can be reservoirs [6, 12]. The detection of infections requires painstaking epidemiological investigation, but these must be undertaken since the infections may have a fatal outcome [10].

Our studies were performed between 24th January and 31st March, 1969.

Material and Methods

The experiments were carried out on the patients of a 24-bed premature baby and 30-bed infant unit. During the period of observation, the beds were practically always occupied. Throat secretion was wiped off the tonsils and the walls of the pharynx. For the removal of nasal discharge likewise a cotton swab was used and, with the aid of a nasal speculum, an attempt was made to wipe both sides as deep as possible. When taking ear secretion, the discharge was removed from the paracental lance taking care that bacteria from the exterior wall of the auditory meatus should not pass into the sample. The test material was at once sent to the laboratory in sterilized sealed test tubes where it was immediately inoculated on blood, chocolate and ordinary agar, as well as into methylene blue eosin culture medium. After 24 hours incubation, there was no difficulty in identifying the bacteria on the basis of colony morphology, colour, odour, Gram staining and biochemical tests.

Antibiotic sensitivity of the strains was studied with the Biotest "A" disk series (Human, Budapest). The sensitivity of 58 pseudomonas and 48 proteus strains was investigated in solid medium with a disk containing 100 μg carbenicillin, and investigation of the quantitative sensitivity to carbenicillin by the tube method in serial dilutions. The concentration which inhibited growth was read after 18 hours incubation. In addition, the antibiotic sensitivity of 8 pseudomonas and 20 proteus standard strains received from the State Institute of Public Health, Budapest, was studied; the sensitivity of these strains coincided with that of the bacteria isolated from the patients in the ward. Thus altogether the antibiotic activity of 66 pseudomonas and 68 proteus strains was investigated. (Table I.)

Results and Discussion

It is well-known that the occurrence of pseudomonas and proteus is more frequent than the diseases caused by them. It would not be right if patients would only be treated on the basis of bacteriological positivity. For that reason, an attempt was made to summarize what we considered an infection caused by pseudomonas or proteus. It were useful if the pathogenic and apathogenic strains could be separated by serological methods; conditions for this have, however, not yet been created in the hospital.

Thus, disease caused by pseudomonas or proteus is meant if

a) the organism is grown in pure culture or dominantly;

TABLE I

Carbenicillin sensitivity of *Pseudomonas aeruginosa* and *Proteus* strains

Carbenicillin concentration in μg/ml	Pseudomonas aeruginosa 66 strains						Proteus 68 strains					
	sensitive		moderately sensitive		resistant		sensitive		moderately sensitive		resistant	
		%		%		%		%		%		%
5	—	—	—	—	66	100	26	38.23	12	17.64	30	44.13
10	—	—	—	—	66	100	41	60.29	10	14.70	17	25.01
20	—	—	—	—	66	100	43	63.23	12	17.54	13	19.03
50	9	13.63	21	31.83	36	54.54	50	73.53	4	5.94	14	20.53
100	53	80.31	9	13.63	4	6.06	66	97.03	—	—	2	2.97
250	63	95.46	—	—	3	4.54						
500	64	96.98	1	1.51	1	1.51						
1000	65	98.49	—	—	1	1.51						
1500	66	100	—	—	—	—						

b) an identical strain is cultivated repeatedly;

c) the infection cannot be identified after adequate treatment, and the patient was cured;

e) it was possible to cultivate the organism post mortem.

Pseudomonas or proteus had not been responsible for the condition if

a) no well-defined disease could be found;

b) even after adequate treatment, an identical organism could be cultivated;

c) a different organism could be identified post mortem;

d) in a mixed flora it was cultivated with other organisms.

During the period of observation, altogether 705 bacteriological investigations were carried out. In 104 infants, the number of investigations amounted to 430 and the corresponding number for 60 premature babies was 275. Bacteriological results are given in Table II.

TABLE II

Bacterial strains isolated from patients

Cultivated strain	Neonatal unit	Premat. baby unit	Total
E. Coli	53	33	86
Staphylococcus aureus haemolyticus	79	47	126
Pseudomonas aeruginosa	43	15	58
Proteus	37	11	48
Klebsiella	14	15	29
Streptococcus pyogenes	5	1	6
Diplococcus PN	4	—	4
Haemophylus influenzae	2	—	2
Fungal growth	5	6	11
Total	242	128	370
Normal flora or sterile	188	147	335
Total	430	275	705

TABLE III

Pseudomonas aeruginosa and *Proteus* strains cultivated, according to the location of the specimen

Test substance	Total No. of tests		Pseudomonas aeruginosa		Proteus	
	premature	neonate	premature	neonate	premature	neonate
Nasal discharge	129	160	5	3	4	3
Throat discharge	131	189	8	13	6	8
Ear discharge	—	49	—	19	—	14
Urine	—	12	—	—	—	10
Other (skin, eye)	9	26	2	8	1	2
Total	269	436	15	43	11	37

Pseudomonas was cultivated from 28 patients in 58 cases, proteus from 21 patients in 48 cases. The cultivated strains according to the location of the specimen taken are listed in Table III. The strains were cultivated most frequently from the ear discharge. From the throat, nose and from urine, they were isolated in approximately identical ratios.

In contrast to our previous observation [3], otitis media has not once been observed in premature babies during the period of observation, and in their urine proteus has never been found; on the other hand, in infants, proteus was discovered in 10 cases.

The antibiotic sensitivity of the investigated pseudomonas and proteus strains is shown in Table IV. All the strains were practically resistant to streptomycin, chloramphenicol, neomycin, tetracyclin and sulphadimidine. The greater half of the strains was sensitive to polymyxin B. The proteus strains were only partly sensitive to neomycin, chloramphe-

TABLE IV

Antibiotic sensitivity of *Pseudomonas aeruginosa* and *Proteus* strains

Antibiotic	Pseudomonas aeruginosa, 66 strains						Proteus, 68 strains					
	sensitive		moder. sensit.		resistant		sensitive		moder. sensitive		resistant	
		%		%		%		%		%		%
Streptomycin	3	4.54	14	21.21	49	74.24	43	63.23	4	5.88	21	30.88
Chloramphenicol	—	—	1	1.51	65	98.49	40	58.82	8	11.76	20	29.42
Neomycin	7	10.60	27	40.90	36	54.54	52	76.47	5	7.35	11	16.17
Nitrofurantoin	—	—	—	—	66	100	11	16.17	13	19.11	44	64.72
Polymyxin	39	59.09	8	12.12	19	28.94	6	8.82	—	—	62	91.18
Tetracyclin	—	—	—	—	66	100	1	1.47	—	—	67	98.53
Sulphadimidine	—	—	—	—	66	100	1	1.47	—	—	67	98.53

nicol and streptomycin; 91% of the strains were resistant to polymyxin B. Cephaloridin, not being effective against pseudomonas and proteus, has not been investigated.

The investigation also covered the nasal and pharyngeal secretion taken from the doctors and nurses working in the wards. From the 46 members of the staff, pseudomonas or proteus was not cultivated in any of the cases. On one occasion *Staphylococcus aureus* was cultivated in 6 cases. Appliances in the unit (incubator, furnishings, oxygen cylinder and pipes, battery of cocks and sink, food and medical instruments, etc.) were also tested; pseudomonas or proteus was not cultivated in any of the cases, while *E. coli* was isolated in three cases, *Staphylococcus aureus* in a single case.

The antibiotic sensitivity of the strains actually reveals the difficulties of treatment. Because of age-specific pecularities of drugs, premature babies and neonates can not be treated with streptomycin and polymyxin in case of danger to life since these drugs have pronounced nephrotoxic and ototoxic properties, particularly for young neonates. For that reason, polymyxin was never administered to premature babies and only exceptionally and for short periods to infants. As to proteus, part of the strains was sensitive to neomycin, chloramphenicol and streptomycin; neomycin is rarely given, particularly in the form of injection, to premature babies and neonates.

Quantitative sensitivity to carbenicillin of 68 proteus and 66 pseudomonas strains is shown in Table I; the overwhelming majority of pseudomonas strains was sensitive to 100 μg/ml and 2/3 of the proteus strains to 50 μg/ml of the drug.

Nine of the 12 patients had purulent otitis; after unsuccessful antibiotic treatment 8 patients had to be operated upon. During the operation bone necrosis was observed in each case. In three cases, carbenicillin treatment failed to ensure bac-

TABLE V

Antibacterial spectrum of Carbenicillin according to KNUDSEN et al. [4]

Organism	Smallest inhibiting dosage microgram/ml-Carbenicillin
Escherchia coli	5.0
Klebsiella aerogenes	250.0
Salmonella typhi	12.5
Shigella flexneri	5.0
Shigella sonnei	5.0
Pseudomonas aeruginosa (111 strains)	12.5—250
Proteus mirabilis	2.5
Proteus morganii	5.0
Proteus rettgeri	2.5
Proteus vulgaris	5.0
Staphylococcus aureus Oxford	0.5
Beta-haemolytic Streptococcus	0.25
Streptococcus faecalis	25.0
Streptococcus pneumoniae	0.5

teriological negativity. The organism disappeared only after the operation.

Table VI also indicates that, in most cases, former premature babies and infants weakened by other disease or rickets were infected by pseudomonas as has been pointed out by other authors [7, 9]. Thus antibiotic treatment can only moderate the symptoms and antrotomy must be carried out. The advantage of carbenicillin was possibly that fatal pseudomonas sepsis has not developed in any of our cases. The ear discharge was mucous, muco-purulent or purulent; the characteristic bluish green purulence only occurred in a single case (F. I., Case 5). MAHNKE [7] also drew attention to the fact that a characteristic bluish green pus is rare in neonates.

In the following, three characteristic cases are described.

Case 1. P. R. neonate was admitted with a fist-size omphalocele. The hernial sac contained intestines and the greater part of the liver. After reconstructive operation, the wound in the abdominal wall suppurated and yielded first proteus and pseudomonas and subsequently pseudomonas in pure culture. Carbenicillin treatment caused moderate improvement but subsequent local carbenicillin treatment ensured a cure.

Case 10. T. L. was admitted when three months old after he had been treated in another institute for 9 days with otitis media and influenza. At admission to our hospital the atrophic infant was in a grave state with bilateral bronchopneumonia and otitis. In the throat swab *E. coli* dominated, from the ear discharge a pure culture of pseudomonas grew repeatedly. The patient died after 9 days. Post mortem, *E. coli* was cultivated from the lungs. It was assumed that the death was due to *E. coli* pneumonia and pseudomonas mastoiditis. The ineffectiveness of carbenicillin in this case could not be evaluated because of the short duration of the treatment.

Case 12. B. J., a premature twin with a birth weight of 1300 g, was admitted when 4 months old with extensive right-sided pneumonia, complicated by grave spastic bronchitis. From the throat and nose discharge proteus was grown repeatedly. Chloramphenicol, cephaloridin and neomycin were ineffective, and so was treatment with steroid. The cultivated proteus was found to be sensitive to carbenicillin. On such treatment, the state improved rapidly; after 8 days, proteus could not be cultivated from the discharge. The further clinical cases are presented on Table VI.

In all cases of pseudomonas infection, the leucocyte count was normal, in agreement with data in the literature. The moderate increase of the ESR did not correspond to the gravity of the disease.

Some attempts were also made with prophylactic carbenicillin treatment of high risk infants. In such infants, kept in a ward where pseudomonas appears it is impossible to calculate whether the symptom-free carriers will contract the disease in a mild or in a serious form. The greatest danger is from otitis, pneumonia, enteritis. In a case the appearance of otitis was followed within 24 hours by fatal meningitis [9]. Polymyxin and gentamicin are toxic to premature babies and neonates while cephaloridin is ineffective against pseudomonas and proteus. Therefore, a trial was made with carbenicillin. Table V. In case of actual or

TABLE VI

Neonates subjected to carbenicillin treatment

No.	Age, sex	Course	Bacteriology	Carbenicillin mg/kg	Duration of treatment	State after treatment
1. P. R.	newborn male	Omphalocele. Reconstruction operation. Wound sepsis	Pseudomonas repeatedly (4×) from wound discharge	150 mg + 0.2% local treatment	6 days	Wound improved on parenteral, healed on local treatment
2. O. Gy.	2 months female	Premature twin A. Haemolytic disease. No incompatibility. Exchange transfusion. Bronchitis, bronchopneumonia	Pseudomonas cultivated 3×	100 mg	7 days	Cured and bacteriologically negative
3. H. I.	9 months male	6 weeks history of otitis. Other antibiotic treatment ineffective	Pseudomonas cultivated 4×	150 mg 150 mg	6 days 7 days	Improved on carbenicillin cure and after operation new carbenicillin treatment
4. K. E.	11 months female	3 months history of otitis. Other antibiotic treatment ineffective	Pseudomonas cultivated 3× from wound discharge	200 mg	9 days	Cure after operation and carbenicillin
5. F. I.	6 months male	Battered child syndrome. Greenish blue thick purulent ear discharge	Pseudomonas 3×. Wound discharge negative	200 mg 150 mg	8 days 6 days	Cure after operation and repeated carbenicillin treatment
6. Z. I.	8 months female	Premature. Haemolytic disease, no incompatibility. Exchange transfusion. Pneumonitis, hepatitis. Otitis for 3 weeks history	Pseudomonas 3×, also from wound discharge	150 mg	8 days	Cure after operation and carbenicillin treatment
7. M. I.	9 months male	4 months history of otitis. Antrotomy. Dystrophy, Septicaemia	Previously repeatedly Proteus Staph. aur. Pseudomonas from wound discharge	150 mg	8 days	Grave septic condition. Did not improve by treatment with other antibiotics. Cure antrotomy & carbenicillin

TABLE VI cont

No.	Age, sex	Course	Bacteriology	Carbenicillin mg/kg	Duration of treatment	State after treatment
8. O. I.	7 months female	4 weeks history of otitis. Rickets	Pseudomonas 2×. Wound discharge sterile	150 mg 150 mg	6 days	Cure after repeated carbenicillin treatment
9. E. A.	7 months male	6 weeks history of otitis. Rubeola	Pseudomonas 3×. Wound discharge sterile	150 mg	6 days	Cure after repeated carbenicillin treatment
10. T. L.	3 months male	Dystrophy. Grave bilateral bronchopneumonia, otitis	Ear discharge Pseudomonas. Throat discharge E. coli. Post mortem lungs E. Coli	200 mg	4 days	Died. No operation in view of grave condition
11. V. L.	2 months male	Premature. Down's syndrome, Atrophy. Pneumonia, relapsing otitis	Repeated Proteus. Wound discharge Proteus	150 mg	6 days	Died. Ear discharge cured
12. B. J.	4 months male	Premature twin B. At 4 months grave bronchopneumonia, spastic bronchitis	Proteus 6× from subglottic secretion	150 mg	9 days	Other antibiotic treatment ineffective Cure after carbenicillin administration

suspected infection by *Klebsiella* or penicillin-resistant staphylococci, the treatment should be supplemented by methicillin, cephaloridin or ampicillin.

Eight high risk premature babies and neonates were prophylactically given carbenicillin in a dosage of 150—200 mg/body weight for 5—6 days (Table VII), without any other antibiotic. Infection and death have not occurred in any of the cases. Since then the favourable effect of prophylactic carbenicillin could be observed in 20 similar patients. The small number of cases and the lack of controls makes it impossible to make further deductions.

The diagnostics and treatment of pseudomonas and proteus infections are problematic and the consequences are incalculable. This is why prevention is so important. At present, the most significant factor of prophylaxis is the strict observation of hygienic rules; in premature baby and neonate wards, a severe hygienic discipline is required. It is important to eliminate overcrowding and to resort to well founded antibiotic treatment. All this presupposes a further improvement in the material and personnel conditions of premature baby and neonate wards.

TABLE VII

Prophylactic carbenicillin treatment of high risk neonates

RDS = respiratory distress syndrome.

After treatment, no pathogen could be grown from the discharges. In Cases 4.5 and 6, Gram-negative organisms were cultivated from tracheal secretion.

Serial No.	Birth weight, sex	Course	Carbenicillin mg/kg	Duration of treatment
1. V. F.	2950 g male	Overdue labour. Massive aspiration of meconium-stained amniotic fluid. Apgar 4.	150	6 days
2. R. R.	2000 g female	Premature birth 12 days after mebrane rupture	175	5 days
3. B. J.	1900 g female	Bleedings during pregnancy. Premature birth 8 days after membrane rupture	200	6 days
4. G. G.	2250 g female	Mother had pyelonephritis. Premature birth 2 days after membrane rupture. Rh isoimmunisation, exchange transfusion	150	5 days
5. B.	3350 g female	Membrane rupture 5 days before delivery. Aspiration of meconium-stained amniotic fluid. Apgar 4.	150	5 days
6. B. A.	4150 g male	Overdue labour (42 weeks). Macerated foetus. Aspiration of meconium-stained amniotic fluid	200	5 days
7. Sz. A.	1900 g male	Premature birth. Aspiration. Resuscitation. RDS	150	6 days
8. E. I.	2300 g male	Twin B. Intrapartum aspiration. Pneumonia. Intracranial haemorrhage. RDS	150	6 days

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