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SENSITIVITY OF HERD STRAINS OF KLEBSIELLA PNEUMONIAE TO ANTIBIOTICS AND ITS TREND

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Abstract

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Sixty nine strains of *Kl. pneumoniae* of almost exclusively bovine origin (milk, nostrils, rectum and skin at the base of the udder) were examined and compared with 72 serotype strains of Danish origin isolated in 1955. Our main interest was focused at the contemporary sensitivity to the antibiotics and evaluation of its trend during the past 25 years. In all experiments the disc method was employed, accomplished by the dilution micromethod for determination of the minimum inhibitory concentration of antibiotics in selected experiments.

The following results were obtained:

1) From the total number of 30 preparations examined in both groups of *Kl. pneumoniae* strains 14 antibiotics showed no effect (bacitracin, cephaloridine, erythromycin, fucidine, lincomycin, methicillin, novobiocin, nystatin, oleandomycin, oxacillin, penicillin, pristinamycin, spiramycin, vancomycin).

2) Therapeutically prospective results were obtained with cephalothin, gentamicin, kanamycin, colistin, neomycin, oxytetracycline, paromomycin, polymyxin B, streptomycin and tetracycline. Seventy five to 97.2 % of strains were resistant to ampicillin and carbenicillin.

3) Among sulphonamides (sulphadimidine, sulphamethoxidine and sulphisoxazole), only sulphamethoxidine proved to be effective.

4) An increase in resistance in newly isolated strains of *Kl. pneumoniae* as compared to the Danish serotype strains from 1955 was only observed in gentamicin and colistin (and this only with the dilution method); the number of resistant strains increased by 13 and 13.5 %, respectively.

Antibiotics, sulfonamides, resistance.

Differences in sensitivity of microbial strains to antibiotics require a close cooperation between the therapist and bacteriological laboratory. The laboratory will test the strains and recommend a selection of suitable preparations, their dosage and will control the course of therapy.

In *Klebsiella* infections, the first and especially the third aspects are of importance, as sensitivity of the etiological agent to widely used cephalothin, kanamycin, streptomycin and others oscillates in rather wide range (Bauer and Seeger 1979), and biological experiments in chickens (Smith 1976) indicate an adaptability of *Kl. pneumoniae* to both chloramphenicol and chemotherapeutic agents trimethoprim and nalidixic acid.

Such conclusions follow from several field studies; e. g. Braman et al. (1973) found out of 46 strains of *Kl. pneumoniae* isolated from bovine mastitis 37 resistant to chlortetracycline, 35 resistant to dihydrostreptomycin and 8 strains resistant to neomycin.

Similar results were reported by Weber et al. (1975) who isolated 148 Kl. strains from placenta, uterine cervix, vagina, praeputium, nasal secretions and faeces of mares and horses. Besides 12 strains resistant to streptomycin, 11 resistant ot chloramphenicol and 9 strains resistant to tetracycline, they found another 18 strains polyresistant to two or all three above-mentioned antibiotics.

With an even wider collection of Kl. strains worked Niazi et al. (1977) who isolated them 105 times from the organs and blood of cattle and pigs, and especially from faeces of calves and uterine cervices of sows. Among these, 54 strains were resistant to streptomycin, 29 to chloramphenicol, 28 to chlortetracycline, 14 to kanamycin and 11 to neomycin whereas all strains were sensitive to gentamicin and polymyxin B

The aim of the present work was not only to accomplish the data on sensitivity of *Kl. pneumoniae* to various antibiotics but also to establish its trend during the past 25 years by means of examination of the available comparative strains.

Materials and Methods

For the experiments two groups of *Kl. pneumoniae* were used:

a) 69 freshly isolated strains of almost exclusively bovine origin (9 strains from udder secretions of dairy cows sufferring from acute parenchymatous mastitis, 20 strains from milk of clinically healthy dairy cows of herds in which Kl.-mastitis was repeatedly diagnosed during the past 3—5 years, 21 strains from nasal secretions, 13 strains from rectal contents and 4 strains from skin of the udder base of cows from permanently healthy herds, 1 strains from a cerebral abscess of a young boar and 1 strain from uterine flushings of a mare sufferring from metritis).

b) Seventy two serotype strains of Danish provenience (Orskov 1955) isolated in 1955.

Each of these strains was examined for sensitivity to antibiotics and sulfonamides in two ways:

1) by the disc method (Hejzlar and Výmola 1962) using the antibiotic discs made by Lachema Brno;

2) by the dilution micromethod using the semi-automatic apparatus Dynatech, Mic 2000.

With the disc method, a total of 27 kinds of antibiotic discs were employed (ampicillin, bacitracin, carbenicillin, cephaloridine, cephalothin, chloramphenicol, colistin, erythromycin, fucidin, gentamicin, kanamycin, lincomycin, methycillin, neomycin, novobiocin, nystatin, oleandomycin, oxacillin, oxytetracycline, paromomycin, penicillin, polymyxin B, pristinamycin, spiramycin, streptomycin, tetracycline and vancomycin) and 3 discs with sulphonamides (sulphadimidine, sulphamethoxydine and sulphisoxazole).

The results were evaluated according to instructions of the manufacturer (published by Lachema 1970); sulfonamides, the inhibitory effect of which was somewhat delayed, were evaluated according to the edge of the full culture growth (Bauer and Sherris 1964).

With the dilution micromethod, we used ampicillin, carbenicillin, cephalothin, chloramphenicol, colistin, gentamicin, kanamycin, neomycin, polymyxin B, streptomycin and tetracycline. The preparations were first diluted in buffered diluting fluids up to 2000 μg/ml, whereas all other dilutions (128, 64, 32, 16, 8, 4, 2, 1 and 0.5 μg/ml) were prepared in Mueller-Hinton's broth. Diluted antibiotics were filled by a semi-automatic dispenser into microtitration plates (0.1 ml per well). The inoculum containing 10^6/ml of microbial cells was prepared by 1-hour incubation of the strains in Brain Heart broth at 37 °C. It was filled by an automatic inoculator. The results were read with aid of an illuminated viewer after an 18-hour incubation at 37 °C. For the finding out of resistant strains data on minimum inhibitory concentrations of antibiotics were used as determined by Matsen and Barry (1974).

Results

Among 27 antibiotics, 14 preparations (bacitracin, cephaloridine, erythromycin, fucidin, lincomycin, methicillin, novobiocin, nystatin, oleandomycin, oxacillin, penicillin, pristinamycin, spriramycin and vancomycin) showed no effect in 97.1 to 100 % *Kl. pneumoniae* strains. Similar results were also obtained with sulfonamides: sulfadimidine and sulfisoxazole inhibited bacterial growth only in 28 to 48 % of strains.

The most important facts and answers to our questions are summarized in Table 1 indicating numbers and percentage of resistant strains. Among antibiotics, therapeutically prospective results were obtained with cephalothin, gentamicin, kanamycin, colistin, neomycin, oxytetracycline, paromomycin, polymyxin B, streptomycin and tetracycline. Resistance to ampicillin and carbenicillin was found in 75 to 92.7 % of strains. With herd strains, therapeutically prospective proved to be sulphamethoxydine in 89.9 % of the respective strains.

Table 1

Resistence to antibiotics and chemotherapeuticals in trial and serotype strains of Klebsiella pneumoniae

Preparation	Disc method			Dilution micromethod		
	Criterion (mm)	Trial stranis (69) Number (%)	Serotype strains (72) Number (%)	Criterion (μg/ml)	Trial strains (69) Number (%)	Serotype strains (72) Number (%)
Antibiotics:						
Ampicillin	4.7 >	69 (100)	72 (100)	32 <	52 (75)	57 (79.2)
Carbenicillin	5.2 >	66 (96.6)	70 (97.2)	32 <	62 (89.8)	67 (93)
Cephalothin	3.5	2 (2.9)	11 (15.3)	32 <	2 (2.9)	15 (20.8)
Chloramphenicol	4.2 >	0 (0)	3 (4.2)	25 <	53 (76.8)	51 (70.8
Colistin	1.7 >	1 (1.45)	0 (0)	32 <	19 (27.5)	10 (13.9)
Gentamicin	3.3 >	0 (0)	0 (0)	6 <	10 (14.5)	1 (1.4)
Kanamycin	3.3 >	2 (2.9)	1 (1.4)	25 <	5 (7.2)	2 (2.8)
Neomycin	3.3 >	0 (0)	0 (0)	10 <	4 (5.8)	3 (5.5)
Oxytetracycline	3.3 >	2 (2.9)	3 (4.2)			
Paromomycin	3.3 >	2 (2.9)	0 (0)			
Polymyxin B	1.7 >	0 (0)	0 (0)	5 <	13 (18.8)	15 (20.8)
Streptomycin	3.3 >	2 (2.9)	11 (15.3)	15 <	9 (13)	11 (15.3)
Tetracycline	3.3 >	3 (4.3)	9 (12.5)	12 <	9 (13)	5 (6.9)
Chemotherapeuticals:						
Sulfadimidine	3.3 >	28 (40.6)	52 (72.2)			
Sulfamethoxydine	2.2 >	7 (10.1)	50 (69.4)			
Sulfisoxazole	3.7 >	29 (42)	49 (68)			

An increase in resistance of freshly isolated strains as against the serotype strains of Danish origin from 1955 was only seen with gentamicin and colistin (and this exclusively with the disc method). The number of resistant strains increased by 13 and 13.5 %, respectively.

Discussion

A therapeutic use of antibiotics and chemotherapeuticals requires an evident sensitivity of the infective agent to the respective preparation. The set of problems is, however, complicated as a portion of the administered antibiotic dose is readily bound to blood plasma proteins (Ziv and Sulman 1972), and its portion reaching the body and tissue fluids may vary from organ to organ. On the other hand, the therapeutic dose may not be needlessly increased as all antibiotics and chemotherapeuticals exert adverse or toxic effects (Huber 1971; Rückstuhl 1976). Regarding these facts, the test discs are saturated with only such amounts of preparations which correspond to their dosage in practice. The dilution micromethod is arranged in a similar way.

In the present work, a good agreement as to the portion of resistant *Kl. pneumoniae* strains was obtained by both methods. Conformity was also obtained in individual examinations. Smaller differences were found only in ampicillin, colistin, gentamicin and polymyxin B, and substantial differences were found with chloramphenicol. It is noteworthy that our data are in good agrement with those on *Kl. pneumoniae* strains from non-pathological material published 13 years ago (Výmola et al. 1967).

Antibiotic treatment of *Kebsiella* infections in cattle seems to be of great advantage as indicated by a remarkable sensitivity of examined *Kl. pneumoniae* strains to selected antibiotics also mentioned by other author (Modr et al. 1969;

Bauer and Seeger 1979). A moderate increase in resistance to colistin and gentamicin should be kept in mind. We do not analyze or discuss this phenomenon for the time being.

Citlivost stájových kmenů Klebsiella pneumoniae na antibiotika a její trend

V práci bylo vyšetřeno 69 kmenů *Kl. pneumoniae* téměř výhradně bovinního původu (z mléka, nosních otvorů, rekta a s kůže při bázi vemena) a srovnáno se 72 sérotypovými kmeny dánského původu z roku 1955. Hlavní zájem se týkal současného rejstříku účinných antibiotik a posouzení možného trendu na tomto úseku za posledních 25 let. V rozsahu všech pokusů bylo použito diskové metody a v nejvýznamnější etapě také diluční mikrometody pro stanovení minimální inhibiční koncentrace antibiotik.

Dosažené výsledky:

1. Z celkového počtu 30 zkoušených preparátů bylo v obou skupinách kmenů 14 antibiotik (bacitracin, cephaloridin, erythromycin, fucidin, linkomycin, methicilin, novobiocin, nystatin, oleandomycin, oxacilin, penicilin, pristinamycin, spiramycin, vankomycin) bez jakéhokoliv účinku.
2. Terapeuticky perspektivní výsledky byly zjištěny u cephalotinu, gentamycinu, kanamycinu, kolistinu, neomycinu, oxytetracyklinu, paromomycinu, polymyxinu B, streptomycinu a tetracyklinu. Vůči ampicilinu a karbenicilinu bylo 75—97,2 % kmenů rezistentních.
3. Ze sulfonamidů (sulfadimidin, sulfamethoxydin, sulfisoxazol) se projevil jako terapeuticky nadějný pouze sulfamethoxydin.
4. Co se týká možného nárůstu rezistence u čerstvě izolovaných kmenů oproti sérotypovým kmenům dánského původu z roku 1955, lze o něm hovořit pouze u gentamycinu a kolistinu (a to jen za použití diluční metody), kde se počet odolných kmenů zvýšil o 13 resp. 13,5 %.

Чувствительность хлевных штаммов Klebsiella pneumoniae к антибиотикам и ее тренд

Авторы обследовали 69 штаммов *Kl. pneumoniae* почти исключительно бычьего происхождения (из молока, носовых отверстий, прямой кишки и с кожи на основании вымени); было проведено их сравнение с 72 серотиповыми штаммами датского происхождения от 1955 г.

Главное внимание уделялось имеющейся в настоящее время чувствительности к антибиотикам и обсуждению возможного тренда в этом отношении за последние 25 лет. В ходе всех опытов использовались дисковый метод и в важнейшем этапе также дилюционный микрометод для установления минимальной ингибиционной концентрации антибиотиков.

Полученные результаты:

1. Из общего количества 30 испытуемых препаратов в обеих группах штаммов было установлено 14 антибиотиков, недающих никакого эффекта (бацитрацин, цефалоридин, эритромицин, фуцидин, линкомицин, метици-

лин, новобиоцин, нистатин, олеандомицин, оксацилин, ценициллин, пристинамицин, спирамицин, ванкомицин).

2. Терапевтически перспективные результаты были получены у цефалотина, гентамицина, канамицина, колистина, неомицина, окситетрациклина, паромомицина, полимиксина В, стрептомицина и тетрациклина. В отношении к ампициллину и карбенициллину 75—97,2 % штаммов оказались резистентными.

3. Изо всех сульфонамидов (сульфадимидин, сульфаметоксидин, сульфисоксазол) в терапевтическом отношении надежным оказался только сульфаметоксидин.

4. Относительно возможного увеличения резистентности у свеже выделенных штаммов в сравнении с серотиповыми штаммами датского происхождения от 1955 года, оно кажется возможным у гентамицина и колистина, (лишь за использования дилюционного микрометода), у которых количество резистентных штаммов увеличилось на 13—13,5 %.

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