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Development of a Typing Method for Epidemiological Analysis of *Klebsiella* Strains based on the Combined Use of Klebocins and Bacteriophages

Key words: Klebsiella, Klebocin, Bacteriophage, Typing, Food, Infection

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Introduction

Klebsiella are important agents of opportunistic infections and isolated from the urogenital- and respiratory tract and from wounds (1). *Klebsiella pneumoniae* was also shown to cause gastroenteritis due to enterotoxins (2, 3). Among Gram-negative bacteria causing nosocomial infections, *Klebsiella* are isolated almost as frequently as *Escherichia coli* (4). Furthermore, the ability of rapid uptake of R-plasmids indicates *Klebsiella* as a particularly serious infectious agent in hospitals (5, 6). Septicaemia and pneumoniae caused by these bacteria are serious diseases with mortality rates up to 50% (7). For this reason, epidemiological surveillance in hospitals focusing on *Klebsiella* is claimed by different authors (8, 9).

Klebsiella is mainly found in the gut of humans and animals (10). Another important reservoir of this microorganism are foodstuffs (2, 11). Different authors postulated contaminated foods to be the source of hospital infections (12–14). To proof this hypothesis however, weakly discriminating methods such as bio- or serotyping were used. Epidemiological analysis of *Klebsiella* can also be done with suceptibility testing to bacteriocins (klebocins), a method which is successfully used until today as shown by a recent publication of *Podschun* and *Ullmann* (15). Bacteriocinotyping is characterized by a solid reproducibility, typability of strains and discriminatory power. However, the last-mentioned parameter can be considerably improved by using both bacteriocins and phages as shown by *Lebek* et al. in typing experiments with *Listeria monocytogenes* (16). Based on the findings of these authors, a method for the epidemiological analysis of *Klebsiella* based on the combined susceptibility to klebocins and phages was developed.

Material and methods

Strains

The screening for strains releasing klebocins and phages was performed with 450 *Klebsiella* isolates made available by six laboratories of medical diagnostics. The strains were isolated from urine, sputum, wound swabs and pleura punctates. After receiving, the strains were subcultured on McConkey agar (Oxoid CM 817) and the species identified with the API 20E system (BioMérieux 20700). For long time storage, several colonies of a pure culture were subcultered on tryptone soya agar (Oxoid CM131) and the bacterial cells harvested with 1 ml of sterile skim milk (10 g powdered milk in 100 ml of distilled water). Cell suspensions were then frozen in cryotubes and stored at –70 °C. Working strains were kept on tryptone soya agar at 4 °C for a maximum of two months.

Selection of klebocin- and phage releasing strains

In preliminary experiments, a small series of *Klebsiella* strains was screened for klebocins or phages. Testing was done with an agar overlay technique (16). Strains releasing phages and/or klebocins were defined as producers and strains susceptible to phages and/or klebocins as indicators. With such isolated producers and indicators, the detection system was adapted and optimised with regard to the size of klebocin inhibition zones and plaque formation. Therefore, the type of growth medium and the mitomycin C concentration were evaluated. The compared growth media were tryptone soya agar, Mueller-Hinton agar (Oxoid CM337), brain heart infusion agar (Difco 0418-01) and plate count agar (Biolife 2145). These media were tested in their original composition and supplemented with yeast extract (Difco 0127-01). As for the inducing agent, titration experiments were performed in the range of 0.5–48 µg mitomycin C/ml. With the optimised test method, 100 *Klebsiella* strains were taken as indicators to screen 200 other strains for klebocin and phage production. Those strains found to have the broadest susceptibility to klebocins and phages were finally used to screen all the 450 available *Klebsiella* strains. Identity between klebocin and phage producing strains was excluded by comparing activity ranges and by considering that a producer strain is immune to its own bacteriocin or phage (17).

Optimised method for combined susceptibility testing to klebocins and phages

For testing, Mueller-Hinton agar supplemented with 0.25% yeast extract was used. Standardised test plates (20 ml agar per Petri dish of 8.5 cm diameter) were prepared the day before the typing experiment was performed. Before being used, plates were opened and dried upside down for 30 min at 37 °C. On the bottom of the test plates, the areas for the inoculation of the producer strains (not more than 12 per plate) were marked. Subsequently, 10 µl of a solution containing 25 µg of

mitomycin C was added onto the agar surface of every marked spot. The mitomycin C solution was freshly prepared from a 400 µg/ml stock solution. Without loss of activity, the stock solution could be stored for three weeks at 4 °C. As soon as the inducing agent was absorbed, the producer strains were inoculated with a single stick of a platinum needle (1 mm gauge) and the plates incubated at 37 °C for 7 h. After incubation, the plates were opened and placed for 1 h upside down on a piece of filter paper (Schleicher und Schuell 2668) which was soaked with chloroform. Before continuing the procedure, traces of chloroform were allowed to evaporate by keeping the slightly open test plates for 1 h at room temperature. In the following step, the strain to be examined for klebocin and/or phage susceptibility was overlaid. For this purpose, cells from 6 colonies of a pure culture were inoculated the day before in tryptone soya broth and incubated overnight at 37 °C. From this preculture, 0.5 ml was added into 7 ml of saline and the cell suspension poured onto the agar surface near the rim of the test plate. It was important that the flow of the cell suspension was in one direction only and that the entire agar surface was moistened. Liquid in excess was decanted and the rim of the Petri dish dried off with an absorptive paper. Subsequently, the test plates were incubated 4 h at 37 °C. If bacterial growth in the overlay was incomplete after this time of incubation, plates were kept at room temperature until a confluent cell layer could be observed. Test plates were finally placed in a refrigerator and kept overnight at 4 °C. The next day, phage plaques and klebocin inhibition zones were analysed.

Typing of Klebsiella strains

Twenty-four klebocin and/or phage producers (see table 3) were used to type all the 450 available *Klebsiella* strains. Reproducibility was tested by typing a series of 10 strains five times on the same day and by typing a series of 100 strains twice on different days.

Results

Optimising the method for detecting klebocins and phages

It was necessary to modify the method used for preliminary screening (15). Among other things, it was shown that the secretion of klebocins is influenced by the concentration of mitomycin C. Sixty-six percent of the tested strains expressed klebocins exclusively under induction. Furthermore, titration experiments showed that a mitomycin C concentration of 25 µg/ml causes an optimal induction without significant inhibition of bacterial growth. As for the four evaluated test media, the largest zones of inhibition resulted on Mueller-Hinton agar supplemented with yeast extract. This medium has also given an optimal plaque formation. In order to have a precise and reproducible phage detection, the standardisation of the over-

laying procedure with the indicators was of great importance. It was also essential that test plates were kept overnight at 4 °C prior to interpretation. By this means, plaque formation was significantly enhanced and with several tested strains, plaques became exclusively visible at refrigerator temperature. It was further noticed that the thickness of the agar layer in the test plates is a critical point. On thin agar layers, *Klebsiella* often secrete more mucus, a factor which can complicate the proper analysis of plaques and inhibition zones.

With specific indicators, a producer can exhibit a klebocin inhibition zone and/or phage plaques as shown in figure 1. The inhibition zones caused by klebocins could be either entirely or partially transparent. The forming of tails by phage plaques was due to floating the test plates from one side to the other with the indicator strain suspension.

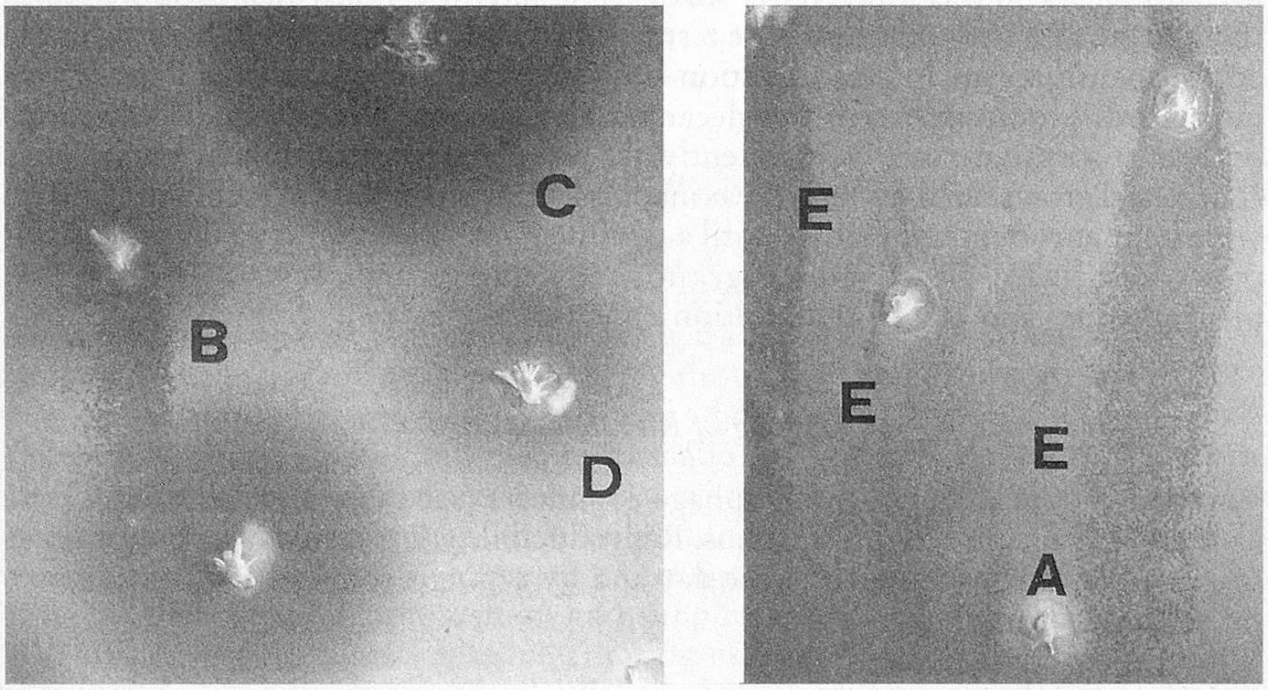

Fig. 1. Sectors of test plates with klebocin inhibition zones and tails of phage plaques
A = Grown producer strain
B = Klebocin inhibition zone and tail of phage plaques
C = Transparent klebocin inhibition zone
D = Semitransparent klebocin inhibition zone
E = Tail of phage plaques

Screening for klebocin producing and detecting strains

When initially screening 200 *Klebsiella* strains with 100 others used as indicators, 23 (11.5%) were found to be klebocin producers. The ranges of activity varied considerably as can be seen in table 1. Two producers were only reactive with 5 strains out of 100 whereas one producer lysed 93 indicator strains.

Regarding the applied indicator strains, highly different patterns of susceptibility were obtained. One strain showed no reaction at all with the 23 klebocin

Table 1. Activity and susceptibility ranges resulting by testing of 23 strains producing different Klebocins with 100 *Klebsiella* indicator strains

Activity ranges of klebocin producers		Susceptibility ranges of klebocin indicators	
Number of klebocin producers	Number of lysed indicator strains	Number of klebocin indicators	Number of detected klebocins
1	93	1	14
1	86	3	13
1	63	1	12
1	53	4	11
2	48	6	10
1	47	8	9
1	45	12	8
1	42	21	7
1	31	17	6
3	18	7	5
2	17	7	4
1	15	8	3
1	12	3	2
3	8	1	1
1	7	1	0
2	5		

producers. Another strain however had the potential to detect 14 klebocins in total. When finally using those indicators with the highest potential of detection to screen all the available 450 isolates, the number of klebocin producers was found to be 61 (13.6%).

Screening for phage producing and detecting strains

When performing the same screening as for klebocins, 49 strains out of 100 were shown to secrete a phage. As table 2 shows, most of the isolated producers were only active against a few indicator strains. However, some producers showed a larger range of activity. In one case, 36 different indicators were lysed. Regarding indicator attributes, 39 out of the 100 used indicator strains showed no lysis with all the 49 phages. On the other hand, two indicators with a high reactivity were found. One of these strains detected 16 and the other 24 different phages. When finally screening all the available *Klebsiella* strains with those indicators found to have the highest potential of detection, 107 different phage producers could be identified.

Table 2. Activity and susceptibility ranges resulting by testing of 49 *Klebsiella* strains producing different phages with 100 indicator strains

Activity ranges of phage producers		Susceptibility ranges of phage indicators	
Number of phage producers	Number of lysed indicator strains	Number of phage indicators	Number of detected phages
1	36	1	24
1	24	1	16
1	17	3	6
1	12	7	5
1	11	6	4
7	8	8	3
6	4	16	2
5	3	18	1
9	2	40	0
17	1		

Application of klebocin- and phage producers for epidemiological typing

For typing, those producers were used i) which showed a broad activity ii) which activity patterns were as different as possible iii) which released, if possible, both a klebocin and a phage (table 3).

When typing all the 450 *Klebsiella* isolates with 24 producers selected according to the above mentioned criteria, 407 types could be demonstrated. 369 of these types were represented by single strains and 37 by two strains each. One type included a group of six strains and three strains were not typable.

Reproducibility of the applied method was examined by typing five times a series of 10 strains. In this experiment, invariable susceptibility patterns with klebocins and phages of the 24 selected producers were observed. Prerequisite for this result was however, that the experiments were performed with a culture derived from a single colony. If cultures from different colonies were used for typing, variable results could be observed. Particularly, this was the case with strains showing a tendency to express different colony morphologies. To exclude this possible factor of variation, we performed further typing with bacterial cells deriving from six colonies. When analysing 100 strains twice with this approach, 88 strains showed repeatable susceptibility patterns. With 12 strains minor discrepancies were observed. Except for five strains, these discrepancies could be eliminated by a third analysis.

To determine the stability of the 24 producers used for typing, 10 subcultures were performed. Each subculture was done with cells from three colonies. Finally, no deletion of either the potential to secrete klebocins nor phages could be observed.

Table 3. Klebocin and phage producing *Klebsiella* strains used for epidemiological typing

Numbers of producer strains	Species according to API 20 E	Location of isolating laboratory	Specimen of strain isolation	Producer characteristics
1	*K. pneumoniae*	Bern	Urine	K/P
2	*K. pneumoniae*	Zürich	Sputum	K/P
3	*K. pneumoniae*	Bern	Urine	K/P
4	*K. pneumoniae*	Bern	Wound swab	K/P
5	*K. pneumoniae*	Bern	Urine	K/P
6	*K. oxytoca*	Bern	Peritoneal fluid	K/P
7	*K. pneumoniae*	Bern	Pleural fluid	K/P
8	*K. pneumoniae*	St. Gallen	Urine	K/P
9	*K. oxytoca*	Bern	Sputum	K/P
10	*K. pneumoniae*	Bern	Pleural fluid	K/P
11	*K. pneumoniae*	Bern	Urine	K
12	*K. pneumoniae*	Zürich	Urine	K
13	*K. pneumoniae*	Genf	Sputum	K/P
14	*K. pneumoniae*	Bern	Sputum	K/P
15	*K. pneumoniae*	Bern	Urine	K/P
16	*K. pneumoniae*	St. Gallen	Urine	K
17	*K. pneumoniae*	Bern	Unknown	K/P
18	*K. pneumoniae*	Bern	Sputum	K/P
19	*K. pneumoniae*	Bern	Blood	K/P
20	*K. oxytoca*	Bern	Sputum	K/P
21	*K. pneumoniae*	Zürich	Sputum	P
22	*K. oxytoca*	Genf	Blood	P
23	*K. pneumoniae*	Bern	Urine	P
24	*K. pneumoniae*	Luzern	Wound swab	P

K = Klebocin P = Phage K/P = Klebocin and Phage

Epidemiological links among the typed strains

The hypothesis that there are epidemiological links between particular types and clinical pictures had to be negated. The surprisingly high number of types which were demonstrated did render it impossible to calculate any correlation.

However, we noticed that six strains, found to be *K. pneumoniae* by testing with the API 20E system, belonged to the same type and had been supplied by the same diagnostic laboratory. By further inquiry, we ascertained that the strains had been isolated over a period of nine weeks from wounds, urine and blood of six different patients. Furthermore, these strains all showed identical antibiograms (data not shown).

Discussion

Adaptation of the detection method

An agar spot method, previously used to detect phages and bacteriocins of *L. monocytogenes* was applied to *Klebsiella* (15). In accordance to *Chugh* et al. (18), we realised that induction with mitomycin C is essential for an effective detection of bacteriocins in *Klebsiella*. As *Chhibber* and *Vadhera* (19), we have seen furthermore that supplementing the growth medium with yeast extract stimulates klebocin secretion. In contrast to these authors however, we observed that Mueller-Hinton agar is superior to tryptone soya agar. With regard to the influence of mitomycin C and the agar medium, we could demonstrate that phages show the same behaviour as klebocins. This fact made it possible to use klebocins and phages for typing in one and the same experiment.

Screening for klebocins and phages and their use for typing

Out of the 450 available *Klebsiella* isolates, we found 61 strains (13.6%) releasing different klebocins. From this result, it could be concluded that klebocin secretion is not frequent enough among *Klebsiella* to be used as marker for typing. On the other hand, susceptibility to klebocins was found to be sufficiently frequent to be used for typing, an observation which was also made by *Edmondson* and *Cooke* (20). The frequency of klebocin producing strains which we found was 2.5 times lower than published by other authors (20, 21). This may be due to the fact that another method of detection was used (18). However, it is worth to be mentioned, that the total number of different klebocins which could be demonstrated is considerably higher than published before (21). The performed screening further resulted in the finding of 107 strains expressing different phages. Out of these strains, 21 were selected for typing purposes. It is not easy to discuss this result because almost no work on phagetyping of *Klebsiella* was done. One of the rare studies in this field is that of *Gaston* et al. (22), who isolated 63 *Klebsiella* phages from sewage and finally used 15 for epidemiological analysis.

Typing method based on the combined use of klebocins and phages

The number of strains in the typing set was limited to 24 for two reasons. First, we expected a sufficiently high discriminating power and second, 24 strains can be inoculated on two agar plates which is, with respect to expenditure of materials, still acceptable for typing elevated numbers of isolates.

When analysing the 450 available *Klebsiella* isolates with the selected 24 producer strains, totally 407 susceptibility patterns could be found. The demonstrated heterogeneity is much higher than published before. *Rennie* and *Duncan* (23) for example applied combined bio-and serotyping on 270 clinical isolates and could demonstrate over 100 different strains. *Podschun* and *Ullmann* (15) recently typed

452 *K. pneumoniae* and *K. oxytoca* strains with a set of eight bacteriocins and found 41 different susceptibility patterns only.

As for the reproducibility of the developed method, it could be shown that variable results can be obtained when typing is done with cultures from different colonies of the same strain. This phenomenon was particularly observed with strains having a tendency to express various colony morphologies. Similar observations were already made by *Simoons-Smit* et al. (24) who investigated the reproducibility of different typing methods based on klebocins. These authors supposed that a varying susceptibility to klebocins is a phenomenon due to changes in the structure of the cell wall which influences the accessibility of klebocin receptors. To avoid this possible source of inaccuracy, typing experiments were consequently performed with cultures derived from six colonies. When typing 100 strains twice like that, a reproducibility of 88% was obtained. By retyping those 12 strains where discrepancies were observed, the reproducibility could be raised to 95%. *Gaston* et al. (22) calculated for their method, based on typing with 15 selected phages, a short term reproducibility of 71%. *Podschun* and *Ullmann* (15), who performed typing with eight klebocin producing strains, do not indicate any value of reproducibility. However, these authors tested every strain at least three times. According to our results, three time testing seems to be also adequate for the combined klebocin/phage typing.

Epidemiological findings obtained by typing of 450 isolates of Klebsiella

The aim of the present study was first of all to screen a large number of *Klebsiella* strains for klebocins and phages and to study their suitability for typing purposes. Nevertheless, we asked the question if there are epidemiological links between types and sites of infection. Analysing our typing results under this angle of view, no correlation could be calculated. *Podschun* et al. (25) examined 176 clinical isolates of *K. pneumoniae* and *K. oxytoca* with plasmid analysis, biochemical reactions, serotyping and antibiotic susceptibility testing and could also not show significant differences between strains of different isolation sites. Furthermore, there was no link between klebocin release and the potential to cause particular infections. This observation is in accordance with that of *Chhibber* et al. (26) who found no significant correlations between particular infections and klebocin types.

When analysing the typing results for other possible correlations, it was noticed that six *K. pneumoniae* strains belonged to the same type. Furthermore, these strains had their origin in a particular hospital. Retrospectively, we ascertained that over a period of nine weeks, the strains had been isolated from wounds, urine and blood of six different patients. This result indicates, that *Klebsiella* strains keep their susceptibility to klebocins and phages over a long period of time. In addition to that, we observed that the potential of producers to release klebocins and phages does obviously not frequently disappear, a fact which is in accordance with the findings of *Simoons-Smit* et al. (24). These authors showed that klebocins are not easily curable and *Chhibber* et al. (27) even presumed a chromosomal integration of the genes encoding klebocins. Another advantage of the developed typing system

is its high discriminatory power. Out of 450 *Klebsiella* isolates, there were only three non typable strains. The typability of > 99% is superior to that of other methods. With a set of eight klebocin producers, *Podschun* and *Ullmann* (15) found 96% of the examined strains typable. However, the power of discrimination of their approach is considerably lower than that of the combined klebocin/phage typing. *Gaston* et al. (22) who worked with phagetyping, found only 76.7% of serologically nontypable strains to be reactive with one or more phages.

As for reproducibility, typability of strains, simplyness of performance and discriminatory power, the developed method seems to be a suitable instrument for epidemiological investigations. With regard to the last-mentioned aspect, *Bannerman* et al. could show combined bactericino- and phage typing to be superior to techniques such as ribotyping, multilocus enzyme electrophoresis and restriction enzyme analysis (28). Recently, *Eisen* et al. (6) successfully investigated an outbreak of multiresistant *K. pneumoniae* with random amplified polymorphic DNA analysis. In future work, it would be worthwile to compare this promising technique with combined bacteriocino- and phage typing. Finally, the developed method fulfils *Rubins* (9) statement that no method to type *Klebsiella* is ideal and that epidemiological characterisation of isolates is best done by using several different marker systems. According to *Ayling-Smith* and *Pitt* (29) however, typing experiments should be performed in specialized laboratories whenever possible, an advice which we fully support.

Conclusions with regard to Klebsiella in foods

With the developed method, a surprisingly high number of different *Klebsiella* strains could be demonstrated. There were almost as many types as clinical isolates. From this finding, it must be concluded that the potential to cause infections is a widely prevalent attribute of *Klebsiella.* As these microorganisms belong moreover to the normal bacterial flora of the human gut, it is highly probable that every individual harbours potentially infectious strains. We therefore presume that the gut itself is the main reservoir for sporadic *Klebsiella* infections and that contaminated food-stuffs are of none or only low relevance. A similar connection was found in the case of *Escherichia coli* mastitis of the cow. It could be shown that a large range of *E. coli* strains can infect the udder and that the gut of the affected animal can be the reservoir of the infectious agent (30). Further epidemiological analysis by combined klebocin- and phage typing will allow to better substantiate the proposed hypothesis. With regard to enterotoxin – producing *K. pneumonia* strains however, foods should be kept in mind. Up to now, this microorganism has not been systematically sought as foodborne enteropathogenic agent. With the nowadays available marker systems however, a more systematic diagnostics and a better epidemiological comprehenson should be possible (2).

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Summary

To develop a combined bacteriocin and phage typing assay on agar for epidemiological analysis of *Klebsiella,* 450 clinical isolates were screened and 61 strains producing different klebocins, and 107 strains producing different phages isolated. Twenty-four of these strains were useable for typing because of their different and broad activity ranges. By analysing all the available 450 strains, 407 different types could be demonstrated. This heterogeneity indicates that the potential of *Klebsiella pneumoniae* and *K. oxytoca* is a widely prevalent attribute. This observation and the fact that *Klebsiella* belongs to the normal fecal flora suggest the gut of the individual to be the main reservoir for sporadic infections and not sources from outside such as food-stuffs. Typing also allowed the identification of six *K. pneumoniae* strains of the same type which were isolated over a period of nine weeks from wounds, urine and blood of six patients from the same hospital. This result shows that the susceptibility to klebocins and phages remains stable over a long period of time. This precondition and the high discriminatory power of combined klebocin and phage typing qualify the developed method for investigations of infection chains and nosocomial outbreaks.

Zusammenfassung

Zur Entwicklung einer kombinierten Bakteriocino- und Phagotypie im Agarplattentest wurden 450 klinische Isolate von *Klebsiella* gescreent und dabei 61 verschiedene Klebocin- und 107 verschiedene Phagenbildner isoliert. Von diesen Stämmen erwiesen sich 24 aufgrund ihres unterschiedlichen und breiten Wirkungsspektrums zur Typisierung speziell geeignet. Bei der Typisierung aller 450 verfügbaren Stämme konnten 407 unterschiedliche Stämme nachgewiesen werden. Diese ausgesprochen grosse Heterogenität weist darauf hin, dass die Fähigkeit, Infektionen zu erzeugen, bei *Klebsiella pneumoniae* und *K. oxytoca* eine grundsätzliche Eigenschaft sein muss. Weil Klebsiellen auch zur normalen Fäkalflora gehören, muss vermutet werden, dass der Darm des Menschen selber die Hauptquelle für sporadische Infektionen ist und Lebensmittel epidemiologisch nur von untergeordneter Relevanz sein dürften. Mit der entwickelten Methode gelang es auch, sechs *K. pneumoniae* Stämme identischen Typs nachzuweisen, die in einem Hospital über den Zeitraum von neun Wochen aus Wunden, Urin und Blut von sechs Patienten isoliert worden sind. Dieser Befund zeigt, dass

die Empfindlichkeit gegenüber Klebocinen und Phagen über längere Zeit unverändert bleibt. Deswegen und wegen der hohen Aufschlusskraft und soliden Reproduzierbarkeit empfiehlt sich das entwickelte Typisierungssystem zur Untersuchung von Infektionsketten und nosokomialen Gruppenerkrankungen.

Résumé

Une méthode basée sue l'action combinée de bactériocines et de phages a été développée pour la classification de souches appartenat au genre *Klebsiella.* 450 souches en provenance du milieu hospitalier ont été analysées au moyen de cette technique afin de trouver des souches productrices de klébocines et de phages. Des 61 souches produisant des klébocines et 107 souches produisant des phages, 24 ont été sélectionnées et utilisées au vue de leur large spectre d'action. Le typage des 450 isolats au moyen de cette méthode a permis de mettre en évidence 407 souches différentes. Cette grande hétérogénéité indique un caractère infectieux largement répandue chez *Klebsiella pneumoniae* et *Klebsiella oxytoca.* Comme *Klebsiella* fait partie de la flore intestinale de l'homme, il est probable que l'intestin lui-même constitue la source principale des infections sporadiques chez l'homme alors que les denrées alimentaires jouent un rôle épidémiologique secondaire. De plus, la méthode utilisée a permis d'identifier dans un même hôpital six souches de *Klebsiella pneumoniae* de type identique. Ces souches ont été retrouvées sur une periode de neuf semaines dans les blessures, l'urine et le sang de six patients. Ce résultat démontre une sensibilité stable des souches durant une longue période vis-à-vis des klébocines et de phages. De ce fait et grâce à son haut pouvoir discriminatoire allié à une solide reproducibilité, la méthode décrite convient au dépistage de chaînes infectieuses et des infections nosocomiales.

Literature

1. *Grimont, F., Grimont, P.A.D.* and *Richard, D.:* The genus *Klebsiella.* In: The Prokaryotes, Balows, A., Trüper, H.G., Dworkin, M., Harder, W. and Schleifer, K.-H. (eds.), 2nd ed., pp. 2775–2796. Springer-Verlag, 1991.
2. *Stiles, M.E.:* Less recognized or presumptive foodborne pathogenic bacteria. In: Foodborne bacterial pathogens, Doyle, M.P. (ed.), 673–733. Marcel Dekker Inc, New York and Basel 1989.
3. *Rennie, R.P., Anderson, C.M., Wensley, B.G., Albritton, W.L.* and *Mahony, D.E.: Klebsiella pneumoniae* gastroenteritis masked by *Clostridium perfringens.* J. Clin. Microbiol. **28,** 216–219 (1990).
4. *Bone, R.C.:* Gram-negative sepsis: a dilemma of modern medicine. Clin. Microbiol. Rev. **6,** 57–68 (1993).
5. *Venezia, R.A., Scarano, F.J., Preston, K.E., Steele, L.M., Root, T.P., Limberger, R., Archinal, W.* and *Kacica, M.A.:* Molecular epidemiology of an SHV-5 extended spectrum β-lactamase in Enterobacteriaceae isolated from infants in a neonatal intensive care unit. Clin. Inf. Dis. **21,** 915–923 (1995).
6. *Eisen, D., Russell, E.G., Tymms, M., Roper, E.J., Grayson, M.L.* and *Turnidge, J.:* Random amplified polymorphic DNA and plasmid analyses used in investigation of an outbreak of multiresistant Klebsiella pneumoniae. J. Clin. Microbiol. **33,** 713–717 (1995).

7. *Bingen, E.H., Desjardins, G., Arlet, F., Bourgeois, F., Mariani-Kurkdijan, P., Lambert-Zechovsky, N.Y., Denamur, E., Philippon, A.* and *Elion, L.:* Molecular epidemiology of plasmid spread among extended broad-spectrum Beta-lactamase producing *Klebsiella pneumoniae* isolates in a pediatric clinic. J. Clin. Microbiol. **31,** 179–184 (1993).
8. *Coovadia, Y.M., Johnson, A.P., Bhana, B.H., Hutchinson, G.R., George, R.C.* and *Hafferje, I.E.:* Multiresistant *Klebsiella pneumoniae* in a neonatal nursery: the importance of maintenance of infection control policies and procedures in the prevention of outbreaks. J. Hosp. Inf. **22,** 197–205 (1992).
9. *Rubin, S.J.: Klebsiella* marker systems. Infect. Contr. **6,** 59–63 (1985).
10. *Selden, R., Lee, S., Wang, W.L.L., Bennett, J.* and *Eickhoff, T.:* Nosocomial *Klebsiella* infections: intestinal colonization as a reservoir. Ann. Int. Med. **74,** 657–664 (1971).
11. *Geiges, O., Stählin, B.* und *Baumann, B.:* Mikrobiologische Beurteilung von Schnittsalat und Sprossgemüse. Mitt. Gebiete Lebensm. Hyg. **81,** 684–721 (1990).
12. *Shooter, R.A., Faires, M.C., Cooke, E.M., Breadon, A.L.* and *O'Farrell, S.M.:* Isolation of *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella* from foods in hospitals, canteens and schools. Lancet **i,** 390–392 (1971).
13. *Casewell, M.W.* and *Phillips, I.:* Food as a source of *Klebsiella* species for colonization and infection of intensive care patients. J. Clin. Pathol. **31,** 845–849 (1978).
14. *Cooke, E.M., Sazegar, T., Edmondson, A.S., Brayson, J.C.* and *Hall, D.: Klebsiella* species in hospital food and kitchens: a source of organisms in the bowel of patients. J. Hyg. Camb. **84,** 97–101 (1980).
15. *Podschun, R.* and *Ullmann, U.:* Bacteriocin typing of *Klebsiella spp.* isolated from different sources. Zbl. Hyg. **198,** 258–264 (1996).
16. *Lebek, G., Teysseire, P.* and *Baumgartner, A.:* A method for typing *Listeria monocytogenes* strains by classification of listeriocins and phage receptors. Zbl. Bakt. **278,** 58–68 (1993).
17. *Jakes, S.K.* and *Lazdunski, C.:* Immunity to colicins. In: Bacteriocins, microcins and lantibiotics, James, R., Lazdunski, C. and Pattus, F. (eds.). Springer-Verlag, Berlin–Heidelberg–New York–Tokyo 1992.
18. *Chugh, T.D., Walia, S.K.* and *Sabharwal, U.:* Bacteriocin (klebocin) sensitivity typing as an epidemiological marker of *Klebsiella pneumoniae*. Indian J. Med. Res. **71,** 195–202 (1980).
19. *Chhibber, S., Dube, D.K.* and *Vadhera, D.V.:* Evidence for chromosomally determined bacteriocin production by *Klebsiella pneumoniae.* Folia Microbiol. **34,** 350–352 (1989).
20. *Edmondson, A.S.* and *Cooke, E.M.:* The development and assessement of a bacteriocin typing method for *Klebsiella.* J. Hyg. Camb. **82,** 207–223 (1979).
21. *Bauernfeind, A.:* Epidemiological typing of *Klebsiella* by bacteriocins. Meth. Microbiol. **16,** 213–224 (1984).
22. *Gaston, M.A., Ayling-Smith, B.A.* and *Pitt, T.L.:* New bacteriophage typing scheme for subdivision of the frequent capsular serotypes of *Klebsiella spp.* J. Clin. Microbiol. **25,** 1228–1232 (1987).
23. *Rennie, R.P.* and *Duncan, I.B.R.:* Combined biochemical and serological typing of clinical isolates of *Klebsiella.* Appl. Environ. Microbiol. **28,** 534–539 (1974).
24. *Simoons-Smit, A.M., Verwij-van Vught, A.M.J.J., Kanis, I.Y.R.* and *MacLaren, D.M.:* Comparison of different methods for bacteriocin typing of *Klebsiella* strains. J. Hyg. Camb. **90,** 461–473 (1983).
25. *Podschun, R., Heineken, P., Ullmann, U.* and *Sonntag, H.-P.:* Comparative investigations of *Klebsiella* species of clinical origin: plasmid patterns, biochemical reactions, antibiotic resistances and serotypes. Zbl. Bakt. Hyg. A **262,** 335–345 (1986).

26. *Chhibber, S., Goel, A., Kapoor, N., Saxena, M.* and *Vadehra, D.V.:* Bacteriocin (klebocin) typing of clinical isolates of *Klebsiella pneumoniae.* Eur. J. Epidemiol. **4,** 115–118 (1988).
27. *Chhibber, S.* and *Vadehra, D.V.:* Effect of medium on the bacteriocin production by *Klebsiella pneumoniae.* Folia Microbiol. **34,** 99–105 (1989).
28. *Bannerman, E., Boerlin, P.* and *Bille, J.:* Typing of *Listeria monocytogenes* by monocin and phage receptors. Int. J. Food Microbiol. **31,** 245–262 (1996).
29. *Ayling-Smith, B.* and *Pitt, T.L.:* State of the art in typing: *Klebsiella spp.* J. Hosp. Infect. **6,** 287–295 (1990).
30. *Baumgartner, A., Nicolet, J.* und *Eggimann, M.:* Plasmidfingerprints von *Escherichia coli* isoliert von Rindermastitiden. Schweiz. Arch. Tierheilk. **125,** 491–496 (1983).

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