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Restriction Fragment Length Polymorphism and Virulence Pattern of the Veterinary Pathogen Escherichia coli O139 : K82 : H1*

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Summary

Escherichia coli O139 : K82 : H1 strains originating from outbreaks and single cases of oedema disease in pigs were characterized by their genomic restriction fragment length polymorphism (RFLP), their virulence pattern, and by the occurrence as well as the genomic distribution of the determinants for hemolysin *(hly)* and verotoxins (shiga-like toxins; *slt*I, *slt*II). Whereas the RFLPs revealed considerable variation among the *E. coli* O139 : K82 : H1 isolates depending the origin and epidemic source of the strains, the virulence gene *slt* II was found to be present in nearly all strains in a particular chromosomal region. Similar to RFLPs, the plasmid profiles are useful for epidemiological analysis.

Zusammenfassung

116 *E. coli* O139 : K82 : H1-Stämme verschiedener Ausbrüche und Einzelfälle der Oedemkrankheit der Schweine wurden hinsichtlich ihres Virulenzmusters, ihrer RFLPs (Chromosomen-Fingerprints) und der Positionsverteilung der Gene (*hly, slt*IIv) für Hämolysin und Verotoxin untersucht. Die ermittelten genomischen Restriktionsmuster (RFLP) zeigten beträchtliche genomische Variationen zwischen den Isolaten, was in guter Übereinstimmung mit der epidemiologischen Herkunft der Stämme stand. Dagegen wurde aber die Determinante für das Verotoxin *IIv* bei allen Stämmen auf dem gleichen Fragment identifiziert, was bedeutet, daß bestimmte chromosomale Bereiche konserviert bleiben. Ähnlich wie die RFLP lassen sich die Plasmidprofile für epidemiologische Aussagen verwenden.

* Dedicated to *Frits Ørskov* on the occasion of his 70th birthday.

Introduction

Oedema disease is one of the very important risks for pigs after weaning. Several O serotypes of *E. coli* (O138, O139, O141 etc.) have been identified as causative agents, however, *E. coli* serovar O139:K82:H1 is ranking first among isolates from outbreaks and single cases (25). This distinct serovar was made responsible for high losses in animal husbandry in many countries, particularly under conditions of industrial farming (15, 25, 30). A continuous high incidence of oedema disease due to *E. coli* O139:K82:H1 scattered over a wide territory speaks either in favour of a particular virulence pattern of this serovar, or in favour of a distinct epidemic situation allowing the spread of a particular strain.

The virulence pattern of *E. coli* O139:K82:H1 has been characterized by the expression of the pathogenic factors – hemolysin *(hly)*, verotoxin II (Shiga-like toxin II; *slt*II or oedema toxin) and adhesins (30). It is unknown, however, to what extent a higher phenotypic expression of these properties or variations in the genotypic make-up may contribute to a particular pathogenic potency of this serovar. Thus we attempted to contribute to a more detailed characterization of *E. coli* O139:K82:H1 strains in respect to their pathogenic potency and epidemiologic significance by the determination of their virulence pattern, restriction fragment length polymorphism and, by long-range mapping of the virulence genes *hly* and *slt*II, also to the phenotypic characterization of *E. coli* strains. The data described in this paper demonstrate a distinct but rather conservative virulence pattern of this serovar and provide evidence of its genotypic heterogenicity. This kind of genotypic variation can be used for epidemiological purposes.

Material and Methods

Bacterial strains. 116 *E. coli* O139:K82:H1 strains were studied. They originated from clinical cases of oedema disease and were isolated post-mortally. The strains had been derived from different outbreaks and single cases scattered over a large territory (Germany, Sweden). They were characterized by standard microbiological methods. A representative collection of these strains has been listed in Table 1.

Determination of phenotypic properties. The determination of the electrophoretic pattern of their multilocus enzymes their outer membrane proteins, and their plasmid profiles, in addition to their serofermentative properties, their phage types and their antibiograms was carried out as described earlier (30). The production and determination of hemolysin was performed according to *Gadeberg* et al. (8). The production of verotoxins was carried out as described by *Stockbine* et al. (26). The quantitative and qualitative characterization of verotoxins by the ELISA techniques was done with polyclonal antibodies raised against purified verotoxins I and II (*W. Beer* and *H. Tschäpe,* unpublished results).

Chemicals, media, and enzymes. Bacteria were grown in L broth without meat extract (22); for toxin production, the syncase medium with and without iron was applied (26). Restriction enzymes were purchased from Gibco, BRL, Eggenstein, Germany and Angewandte Gentechnologie Systeme GmbH, Heidelberg, Germany, and low melting agarose type I from Biometra, Portland, ME, USA. All other chemicals were obtained from Sigma, Frankfurt, Germany.

Pulsed field electrophoresis. Genomic DNA used for pulsed field electrophoresis was isolated according to *Grothues* and *Tümmler* (10). Agarose blocks were equilibrated in restriction enzyme buffer on ice for 3 h. Cleavage with *Xba*I and *Sfi*I restriction enzymes, respectively, was performed according to the manufacturer's instructions. Elektrophoresis

Table 1. Properties of *E. coli* O139:K82:H1 isolates[1]

Nr.	Strains	OMP[2]	ET[3]	Bio-type[3]	Plasmids (Md)[4]	Phage pattern[5]	Antibiogram	Virulence properties[6] Hly	*hly*	SltI	*sltI*	SltII	*sltII*
Outbreak Nordhausen													
I	1) W1891	6	1	2	65,48,36,3.0,2.8	1,3,5,21,30	CmSmSuTcKmTp	+	+	−	−	+	+
	2) W2016	6	1	2	65,48,36,3.0,3.8	1,3,5,21,30	CmSmSuTcKmTp	+	+	−	−	+	+
	3) W2007	6	1	2	65,48,36,3.0,2.8	1,3,5,21,30	CmSmSuTcKmTp	+	+	−	−	+	+
II	4) W2822	6	1	2	36,3.0,2.8	6,7, 21,30	CmSmSu	+	+	−	−	+	+
	5) W1633	6	1	2	36,3.0,2.8	6,7, 21,30	CmSmSu	+	+	−	−	+	+
	6) W1629	6	1	2	36,3.0,2.8	6,7, 21,30	CmSmSu	+	+	−	−	+	+
III	7) W2824	6	1	2	65,48,36,3.0,2.8	1,3,5,21,30	CmSmSuTc	−	+	−	−	+	+
IV	8) W2821	6	1	2	65, 36,3.0,2.8	1,3,5,21,30	CmSmSuTc	+	+	−	−	+	+
Outbreak Goppeln													
	9) W6001	1	1	1	36,8.0,3.4	n.t.[7]	sensitive	+	+	−	−	+	+
	10) W6002	1	1	1	36,8.0,3.4	n.t.	sensitive	+	+	−	−	+	+
	11) W6003	1	1	1	36,8.0,3.4	n.t.	sensitive	+	+	−	−	+	+
Outbreak Sweden													
	12) W6014	6	1	1	60,36,8.0,2.6	n.t.	SmTc	+	+	−	−	+	+
	13) W6075	6	1	1	60,36,8.0,2.6	n.t.	SmTc	+	+	−	−	+	+
Outbreak Nebelschütz													
	14) W5995	6	1	2	40,36,7.5,3.5	1,13,21,30	CmSmSuTcTp	+	+	−	−	+	+
	15) W5997	6	1	2	36,7.5,3.5	1,13,21,30	sensitive	+	+	−	−	+	+
	16) W9598	6	1	2	36,7.5,3.5	1,13,21,30	sensitive	+	+	−	−	+	+
	17) W6000	6	1	2	40,36	n.t.	CmSmTuTc	+	+	−	−	+	+
Outbreak Kodersdorf													
	18) W2830	6	1	2	65,36,2.4	11,19	SmTc	+	+	−	−	+	+
	19) W2833	6	1	2	65,36,2.4	11,19	SmTc	+	+	−	−	+	+
Outbreak Soenitz													
	20) W2840	6	1	2	65,36,7.0,3.0,2.8	1,3,5,30	SuSmTc	+	+	−	−	+	+
	21) W2841	6	1	2	65,36,7.0,3.0,2.8	1,3,5,30	SuSmTc	+	+	−	−	+	+
Outbreak Gotha													
	22) W6018	6	2	3	36,2.5,2.4	1,3,5,30	CmSmSu	+	+	−	−	+	+
	23) W6019	6	2	3	36,2.5,2.4	1,3,5,30	CmSmSu	+	+	−	−	+	+

[1] Representative collection of the strains investigated.
[2] Determined according to *Achtman* et al. (2).
[3] See *Wittig* et al. (30).
[4] Size of plasmids in Md. (Megadalton). The plasmids of strains 1–8 (outbreak Nordhausen) have been typed in detail: 65 Md (IncF2, SmSuTc), 48 Md (IncX, SmSuKmTp); 36 Md (IncN, Hly); 3.0 Md (Inc?, CmSm); 2.8 Md (cryptic).
[5] According to *Wittig* et al. (30).
[6] Hly – phenotype for hemolysin production; *hly* – genotype for hemolysin production
SltI,II – phenotype for shiga-like toxin I,II production, *sltI*,II – genotype for shiga-like I,II production (sltII is in fact sltIIv).
[7] n.t., not tested
Abbreviations: Cm – chloramphenicol; Sm – streptomycin; Su – sulfonamide; Tc – tetracycline; Km – kanamycin; Tp – trimethoprim

was carried out on a CHEF DrII equipment (Biorad, Richmond, CA, USA) at 14 °C in 1% agarose gels (0.5 × TBE), for 20 h at a constant voltage of 200 V. Pulse times are given in each figure legend. As size markers, lambda concatemers (Pharmacia, Freiburg, Germany) and yeast chromosomes (*Saccharomyces cerevisiae* YPH 149, Biometra, Portland, ME, USA) were used.

DNA dot hybridization, Southern hybridization and generation of DNA probes. The DNA-dot procedure was used for rapid detection of the presence of the virulence genes for hemolysin (*hly*) and verotoxin (*slt*I, *slt*II) as described by *Maniatis* et al. (17). Southern hybridization was performed as described earlier (27). The DNA probes (Tables 2) used were generated from plasmid pANN215 (18, 22) as a 3.2 kb *Hind*III fragment (*hly*), from plasmid pIE966 △ P as a 850 bp *Sma*I/*Pst*I fragment (*slt*II), and from plasmid pVP48 △ P as a 850 bp *Sma*I/*Pst*I fragment (*slt*II), and from plasmid pVP48 △ P as a 750 bp *Hind*III-*Bgl*II fragment (*slt*I), respectively. The insert DNA of plasmids pIE966 and pVP48 originated from the phages W34 and J1, respectively (26). DNA techniques were performed according to protocols of *Maniatis* et al. (17). Radioactive labelling was carried out by the random priming technique (7) with a kit purchased from Boehringer (Mannheim, Germany).

Results

Strain subdivision and phenotypic properties

E. coli O139:K82:H1 isolated post-mortally from pigs suffering from oedema disease were subdivided by means of ET type, outer membrane protein pattern (OMP), serofermentative methods, plasmid profiles, phage typing and antibiogram. According to the OMP and ET pattern, all isolates might have been related members belonging to one clone (Table 1), however, according to plasmid profiles, phage typing and antibiogram the isolates from various outbreaks were different and could be discriminated with respect to their origin (outbreak or geographical source) whereas the isolates from the same outbreak were closely related or identical. However, bacterial isolates from one severe and long lasting outbreak (Nordhausen) that ought to be regarded as epidemiologically related, revealed considerable differences in the plasmid profile (Table 1). Plasmid typing, however, demonstrated that some of the plasmids were identical and that different subclones originated from plasmid transfer or deletions.

Virulence properties

All *E. coli* O139:K82:H1 strains were identified as producers of hemolysin and verotoxin II (variant), but not of verotoxin I (Table 1). Quantitative differences, among the strains investigated could not be detected or remained within the variation ranges of the methods (data not shown). The presence or absence of the corresponding genes was confirmed by means of DNA dot-blot hybridization with the DNA probes for *hly*, *slt* I and *slt* II (see Table 2). With the exception of one particular strain (W2824), all strains exhibited identical phenotypes and genotypes.

Restriction fragment length polymorphism (RFLP) of the genome of E. coli O139:K82:H1

In a further attempt, we established the *Xba*I and *Sfi*I genomic profiles of the strains. The determination of RFLP patterns revealed considerable differences in the genomic structure of the *E. coli* O139:K82:H1 strains (e.g. Nordhausen, Nebelschütz, Goppeln, Sweden; see Figs. 1, 2A). Strains from the epidemic outbreak in Nordhausen

Table 2. DNA probes

Plasmid	Properties	Probe	Reference
pANN215	*hly*A Cm^r	3.2 kb *Hin*dIII	18
pIE966 P	*slt*II Tc^r	850, bp *Sma*I-*Pst*I	26
pVP48 P	*slt*I Tc^r	750 bp *Hin*dIII-*BGl*II	26

(Fig. 1, lanes 1–8) exhibited highly related fragments but different patterns. These differences were shown to be due to the different plasmid pattern exhibited by these isolates (see Table 1). In summary, the RFLPs of *E. coli* O139 : K82 : H1 indicated genetic and therefore epidemiological relationships similar to plasmid profiles and phage types.

Identification of the position of the genes hly and sltII in the genome of E. coli O139 : K82 : H1 strains

Using DNA probes for the two virulence genes *hly* and *slt*II, a further characterization of the *E. coli* O139 : K82 : H1 strains was achieved. The gene *slt*II (in reality *slt*IIv)

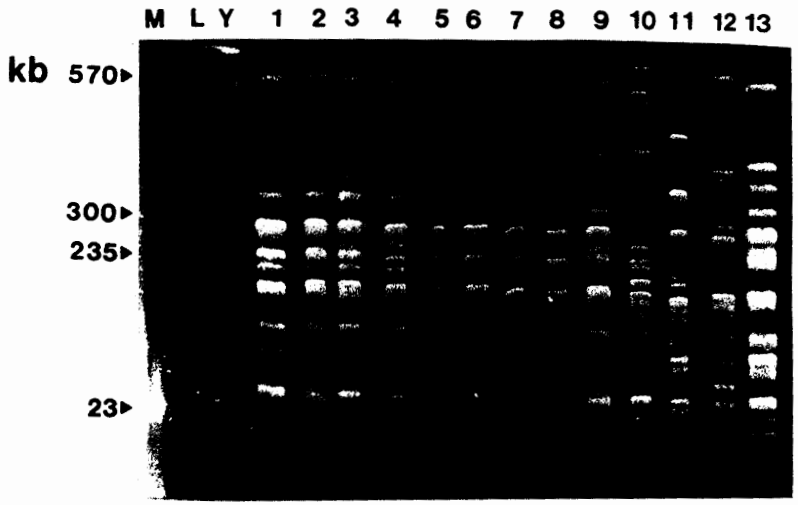

Fig. 1. *Xba*I cleavage pattern of genomic DNA from *E. coli* O139 strains. Strains: 1) W 2016; 2) W 1891; 3) W 2007; 4) W 2822; 5) W 1633; 6) W 1629; 7) W 1824; 8) W 2821; 9) W 5995; 10) W 5997; 11) W 6000; 12) W 6002; 13) W 6014. Strains were derived from the outbreaks Nordhausen (1–8), Nebelschütz (9–11), Goppeln (12), and Sweden (13). Electrophoresis was run with an increasing pulse time from 20–40 sec. Yeast chromosome (lane Y), and lambda concatemers (lane L) were used as high molecular weight DNA standards. *Hin*dIII-cleaved lambda DNA was separated in lane M.

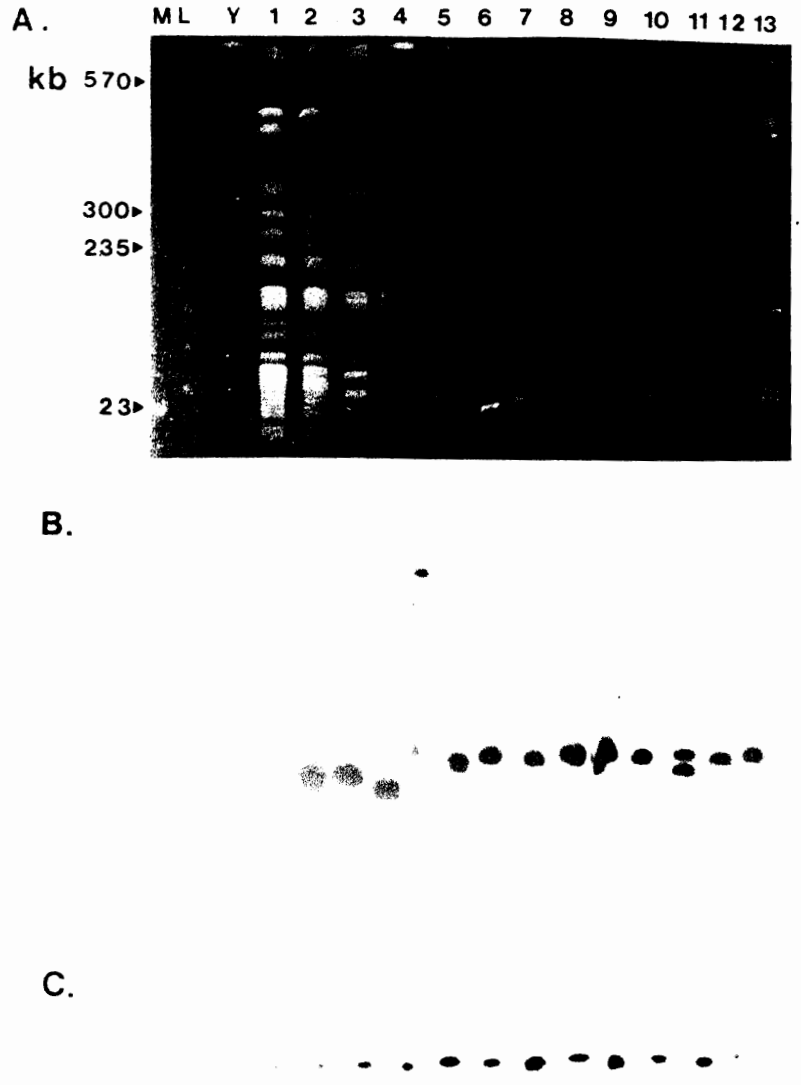

Fig. 2. *Sfi*I cleavage pattern of genomic DNA from *E. coli* O139 strains (A), and Southern hybridization to the *hly* (B), and *slt*II (C) specific DNA probes. Strains: 1) W 2016; 2) W 1891; 3) W 2007; 4) W 2822; 5) W 1633; 6) W 1929; 7) W 1824; 8) W 2821; 9) W 5995; 10) W 5997; 11) W 6000; 12) W 6002; 13) W 6014. Strains were derived from the outbreaks Nordhausen (1–8), Nebelschütz (9–11), Goppeln (12), and Sweden (13). Electrophoresis was run with an increasing pulse time from 15–40 sec. Yeast chromosomes (lane Y), and lambda concatemers (lane L) were used as high molecular weight DNA standards. *Hind*III-cleaved lambda DNA was separated in lane M.

was found in 12 of the 13 strains on the same digestion fragment of nearly 400 kb when using *Sfi*I cleavage (Fig. 2, panel C). In contrast, the hybridization pattern of *hly* was found to be somewhat different with fragments ranging from 60–80 kb (Fig. 2 panel B). One strain exhibited a DNA fragment of 500 kb hybridizing with an *hly*-specific gene probe (Fig. 2 B, lane 4).

Discussion

The study of 116 *E. coli* O139 : K82 : H1 strains isolated from pigs which had died from oedema disease has shown identity or very close relationship between the isolates with respect to their O, K, H antigens as well as to their electrotypes (multilocus enzymes, OMP proteins), however, a diversity with respect to their antibiotics resistance pattern and phage type as well as to their plasmid profiles (30). When applying chromosomal fingerprinting techniques such as RFLP determination, the data achieved throughout this study confirmed the diversity of the *E. coli* O139 : K82 : H1 strains. This diversity goes along with the epidemiological findings; from each epidemic focus (e.g. one farm), bacterial isolates of unique properties or identical complex types were identified, however, strains from different farms were found to be of different genotypes.

The virulence gene *slt*II (in reality *slt*v) was shown to map on identical *Sfi*I digestion fragments in 12 of 13 strains investigated. The overall presence of the toxin gene *slt*II identified in the O139 strains corresponds to the typical virulence picture of the illness (30) and it is rather likely that these fragments are rarely subject to genotypic variations. In contrast, the genes coding for the α-hemolysin of *E. coli* were located on DNA fragments of different length which may reflect the fact that *hly* genes were found on plasmids as well as on chromosomes of strains (12, *Bender* and *Tschäpe,* unpublished data).

The data described here therefore confirm the epidemiological hypothesis that strains originating from one epidemic focus ought to be genetically homogeneous (20, 23, 28). The epidemic spread of one particular strain among a sensitive human or animal population is indicated by genetic identity of the bacterial isolates from clinical cases. Thus the hypothesis "one clone - one epidemic focus" was cloned and introduced into the laboratory analysis of epidemic outbreaks and single cases (20). However, the therm "clone" has been variously defined and used to describe evolutionary, taxonomical or even epidemiological relatedness (1, 2, 6, 13, 22–24). Most of these authors agree with the definition that the term clone should refer to a group of genetically identical or nearly identical cells that owe their similarity to recent descent from a common ancestral cell. However, from an epidemiological point of view this definition needs some modification. A given bacterial species can be regarded as a community of cell lines (24) that are largely independent with respect to their chromosomal genes but which exchange and share a large range of extrachromosomal elements. Even these elements do qualify very often a strain as being harmful for a human or an animal population. Such epidemic strains therefore can be discriminated very often by their plasmid content (28). This implicates that also extrachromosomal elements are especially important for labelling and for detecting epidemic strains. It is interesting to note that genotypic variations such as plasmid content or chromosomal rearrangements can also be detected by inspecting the position of various genes within the genome.

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References

1. *Achtman, M.* and *G. Pluschke:* Clonal analysis of descent and virulence among selected *E. coli.* Ann. Rev. Microbiol. 40 (1986) 185–210
2. *Achtman, M., A. Mercer, B. Kusecek, A. Pohl, M. Heutzenroeder, W. Aaronson, A. Sutton,* and *R. P. Silver:* Six widespread bacterial clones among *E. coli* isolates. Infect. Immun. 39 (1983) 315–335
3. *Appel, E., A. Heer, G. Mickwitz, S. Aleksic, H. Rüssman, T. Meyer* und *H. Karch:* Vorkommen und Bedeutung von Verotoxin-(*Shigella*-like-Toxin) produzierenden *Escherichia coli*-Stämmen, beim Schwein. Tierärztl. Umschau 44 (1989) 410–420
4. *Baumgartner, A.* und *J. Nicolet:* Plasmidgehalt von schweinepathogenen *E. coli*-Erregern von Diarrhoe und Enterotoxämie. Schweiz. Arch. Tierheil. 125 (1983) 497–506
5. *Bertschinger, H. U., U. Jucker, H. P. Pfirter,* and *J. Pohlenz:* Role of nutrition in the pathogenesis of porcine *E. coli* enterotoxaemia. Ann. Rech. Vet. 14 (1983) 469–472
6. *Dykhuizen, D. E., S. A. Sawyer, L. Green, R. D. Miller,* and *D. L. Hartl:* Joint distribution of insertion elements IS4 and IS5 in natural isolates of *Escherichia coli.* Genetics 11 (1985) 219–231
7. *Feinberg, A. P.* and *B. Vogelstein:* A technique for radio-labelling DNA restriction endonuclease fragment to high specific activity. Anal. Biochem. 132 (1983) 6–13
8. *Gadeberg, O. V., J. Hacker,* and *I. Ørskov:* Role of hemolysin for the in vitro phagocytosis and intracellular killing of *E. coli.* Zbl. Bakt. 271 (1989) 205–213
9. *Gammon, V., P. C. L. Gyles,* and *R. W. Priedship:* Characteristics of verotoxinogenic *E. coli* from pigs. Can. J. Vet. Res. 52 (1988) 331–337
10. *Grothues, D.* and *B. Tümmler:* Genome analysis of *Pseudomonas aeruginosa* by field inversion gel electrophoresis. FEMS Microbiol. Lett. 48 (1987) 419–422
11. *Karmalis, M. A.:* Infection by verocytotoxin producing *E. coli.* Clin. Microbiol. Rev. 2 (1989) 15–38
12. *Hacker, J.* and *C. Hughes:* Genetics of *Escherichia coli* hemolysin. Curr. Top. Microbiol. Immunol. 118 (1985) 139–162
13. *Hartel, D. L., D. E. Dykhuizen,* and *D. Berg:* Accessory DNAs in the bacterial gene pool: playground for evolution. CIBA Found. Symp. 102 (1983) 233–245
14. *Hull, S., J. S. Bieler,* and *R. A. Hull:* Restriction fragment length polymorphism and multiple copies of DNA sequences homologous with probes for P fimbriae and hemolysin. Can. J. Microbiol. 34 (1988) 307–311
15. *Jahn, S.* und *E. Uecker:* Ökonomische Untersuchungen zur Koli-Enterotoxämie des Schweines. Mh. Vet.-Med. 42 (1987) 769–771
16. *Lai, E., B. W. Birren, S. M. Clark, M. I. Simon,* and *L. Hood:* Pulsed field electrophoresis. Biotechniques 7 (1989) 34–42
17. *Maniatis, T., E. F. Fritsch,* and *J. Sambrook:* Molecular cloning. Cold Spring Harbor Laboratory, Cold Spring Harbor/NY (1982)
18. *Noegel, A., U. Rdest,* and *W. Goebel:* Determination of the function of hemolysin plasmid pHly152 of *Escherichia coli.* J. Bact. 145 (1981) 233–247
19. *Ochmann, H., T. S. Whittam, D. A. Caugant,* and *R. K. Selander:* Enzyme polymorphism and genetic population structure in *Escherichia coli* and *Shigella.* J. Gen. Microbiol. 129 (1983) 2715–2726
20. *Ørskov, F.* and *I. Ørskov:* Summary of a workshop on the clone concept in the epidemiology, taxonomy and evolution of the *Enterobacteriaceae* and other bacteria. J. Infect. Dis. 148 (1983) 346–357

21. *Ørskov, F.* and *I. Ørskov:* Serotyping of *E. coli.* In: *R. Bergan* (ed.), Methods in Microbiology, Vol. 14, pp. 43–112. Academic Press, London (1984)
22. *Ott, M., L. Bender, G. Blum, M. Schmittroth, M. Achtman, H. Tschäpe,* and *J. Hacker:* Virulence patterns and long range genetic mapping of extraintestinal *E. coli* K1, K5 and K100 isolates: Use of the pulsed field electrophoresis technique. Infect. Immun., submitted (1991)
23. *Rische, H., H. Tschäpe,* and *W. Witte:* On the clone concept in epidemiology and clinical bacteriology. In: *S. Mitsuhashi, V. Krecmery, M. Antal, L. Rosival* (eds.). Transferable antibiotic resistance, pp. 101–107. Avicenum Med. Press, Prague and Springer-Verlag, Berlin–Heidelberg–Tokyo (1984)
24. *Selander, R. K., D. A. Caugant,* and *T. S. Whittam:* Genetic structure and variation in natural populations of *E. coli.* In: *F. C. Neidhardt* (ed.), *E. coli* and *S. typhimurium,* pp. 1625–1648. Washington/DC (1988)
25. *Sojka, W. J.: Escherichia coli* in domestic animals and poultry. Commonwealth Agricult. Bureaux Farnham Royal, Bucks./U.K. (1965)
26. *Strockbrine, N. S., L. R. M. Mawques, J. W. Newland, H. R. Smith, R. K. Holmes,* and *A. D. O'Brian:* Two toxin converting phages from *E. coli* O157 : H7 strain 933 encode antigenically distinct toxins with similar biological activities. Infect. Immun. 53 (1985) 135–140
27. *Southern, E. M.:* Detection of specific sequences among DNA fragments separated by electrophoresis. J. Molec. Biol. 98 (1975) 503–517
28. *Tietze, E.* and *H. Tschäpe:* Plasmid pattern analysis of natural bacterial isolates and its epidemiological implications. J. Hyg. 90 (1983) 475–488
29. *Willshaw, G. A., H. R. Smith, S. M. Scotland, A. M. Field,* and *B. Rowe:* Heterogenicity of *E. coli* phages encoding verocytotoxins; comparison of cloned sequences determining Vt1 and VT2 and development of specific gene probes. J. Gen. Microbiol. 133 (1987) 1309–1317
30. *Wittig, W., H. Tschäpe, V. Thurm,* and *G. Seltmann:* Complex typing of *E. coli* O139 : K82 : H1 strains causing oedema disease in the GDR. Arch. Exp. Vet.-Med. (1991) in press

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