Epidémiol. santé anim., 1997, 31-32

COMPLEX TYPING OF SALMONELLA ENTERITIDIS AND SALMONELLA TYPHIMURIUM AS A TOOL FOR THE EPIDEMIOLOGICAL SURVEILLANCE OF SALMONELLOSIS FROM 1976-1996 IN GERMANY

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Le typage bactériophagique est actuellement la méthode de laboratoire la plus utile pour estimer une possible association épidémiologique entre les souches de S. typhimurium et S. enteritidis. Les souches des deux sérovars (nous typons 5000 à 8000 par an) sont isolées d'échantillons d'origine humaine, alimentaire, animale, et environnementale. Nous avons utilisé plusieurs systèmes de typage phagique (Felix and Callow, Lilleegen, Anderson) pendant plus de 20 ans pour S. typhimurium.
Actuellement, un clone de S. typhimurium DT 104 est au second rang de prévalence des types de salmonelles chez l'Homme en Allemagne. Il a été isolé initialement chez les bovins et les porcs. La pluri-résistance de ces souches revêt une grande importance. Vis-à-vis de l'ampicilline, chloramphénicol, streptomycine, sulfamides et tétracyclines elle est localisée au niveau des chromosomes. Les résultats classiques et moléculaires seront discutés.
Nous avions commencé le typage de la souche S. enteritidis à l'aide d'un système développé en Europe de l'Est (Laszlo) puis ultérieurement par un système de typage international. Les souches S. enteritidis de type phagique 4/6 étaient associées aux élevages de poules pondeuses et de poulets.

SALMONELLOSIS IN GERMANY

Salmonellosis is to be regarded as a general public health problem in Europe. In both parts of Germany Salmonellosis have increased during the 70s and 80s. Thus the number of cases of Salmonellosis reached its highest level in 1992. The incidence declined in 1993 and remains approximately on this reduced level also in 1994/1995. However, the incidence rate slightly increased again in 1996.
The main question of epidemiological and ecological purposes is the characterization of the Salmonella strains as infectious agent, that means to analize the biological properties, the reservoirs and the transmission route.
In comparison to the majority of Salmonella serovars S. Typhimurium and S. Enteritidis seem to differ in the epidemiological point of view. Due to results of long term studies all over the world both the serovars are the most frequent in infections of humans. While the other serovars mostly accumulate at certain intervals or in certain areas for a short time only, we observed a persistancy of both S. Typhimurium and S. Enteritidis strains.
The cooperation between the departments of human medicine and veterinary medicine in the field of surveillance concerning S. Typhimurium in the former GDR (East Germany) was a good opportunity to observe the distribution of phage types and biochemical types of those serovars within the population of different animals and human beings. Since 1990 Salmonella strains were typed in two reference centres in Germany. All of S. Typhimurium strains and the most of S. Enteritidis strains were typed in Wernigerode.

SALMONELLA TYPHIMURIUM

The long term study of phage typing of S. Typhimurium from 1974 - 1996 show us the up and down of different phage types. Over this period the application of phage typing (annually 5000-8000 strains) as epidemiological tool have proved to be very useful in epidemiological investigations. We observed in the 70s the phage type DT204 which dominated as an clone for at least 8 years. These strains were first isolated from cattle and beef products. Later one, this clone was also isolated from other animals including poultry. A new variation of S. Typhimurium called DT104 with chromosomally encoded multiresistance was also observed in the earlier 90s in cattle and pigs. All conventional and molecular methods confirm the distribution of S. Typhimurium DT104 as one clone.
Furthermore, a subdivision of distinct phage types was possible by analysis of the O5 antigen detection, biotyping, fimbriation, plasmid pattern, siderophore pattern and by outer membrane profiles. The bacteria from food poisoning, nosocomial infection in children hospitals and different animals could be subdivided into five different groups.

Group 1 : The strains were mostly isolated from food poisoning and animals.
These strains belonged to different phage types. They harbour a 60 MDa plasmid and sometimes of cryptic plasmids. The epidemic strains exhibited most antibiotic resistance like DT204 in the 70s and 80s and like DT104 in the 90s type-1 fimbriae and some different siderophore pattern.

Group 2 : These strains were isolated from nosocomial infections.
We received them from different countries (USSR, Mongolia, Hungary and Germany). The strains were untypeable with both the Felix and Callow and the Lilleengen system and characterized as Anderson phage type DT208. All strains were unable to use d-tartrate and miss the 60 MDa S. Typhimurium plasmid. The large

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plasmids detected in these strains belonged to an other incompatibility group called IncFI$_{me}$. These plasmids were firstly isolated in Middle East and carry drug resistance determinants. Additionally, they encode phage restriction capacity. The siderophore pattern was different, but all strains produced aerobactin.
We were very interested in the occurrence of DT208 in animals and found some Tc resistant strains in pigs. They carry also a 95 MDa plasmid with aerobactin determinants.

Group 3 : Probably ancestor of group 2 strains.
Among them, a group of S. Typhimurium strains of the biochemotype 3 was detected not possessing the 60 MDa plasmid. Those strains were sensitive to antibiotics and belonged to the Anderson phage types DT10 or RDNC. They produced enterobactin only. It might be possible that these strains are the natural related strains of nosocomial strains.

Group 4 : FIRN strains.
The strains in this group are not equipped with fimbriae, don't produce acid from inositol and rhamnose and are called by Duguid and Old as FIRN strains. FIRN strains are particularly common in avian hosts, song bird and seegulls. Four phage types and three biochemotypes were detected. The 60 MDa plasmid was present and all strains produced enterobactin.

Group 5 : The last group contains strains from pigeon Salmonellosis. All strains belonged to the variety „Copenhagen". We found two phage types DT2 and DT99 only but three biochemotypes. All strains have the 60 MDa plasmid and produced small amounts of enterobactin. These strains were very rarely in human infections.

The OMP-profiles of the most phage-types of S. Typhimurium behaved unique. However, all strains of phage-type DT8 produced an additional 54 kDa OMP and all strains of phage-type DT10 an additional OMP of 39 kDa.

SALMONELLA ENTERITIDIS

In former time S. Enteritidis has been subdivided in 4 biochemical groups: S. Enteritidis var. jena, var. chaco, var. essen, var. danysz. Prior to 1984 strains of Salmonella Enteritidis occurred not frequently in Germany. However, in 1972/73 an increase in the incidence of S. Enteritidis strains was recorded. These strains originated from calves and were transported via beef to consumers. Since 1972 there was a significant increase in S. Typhimurium, S. Enteritidis disappeared widely. Suddenly, in 1984/85 outbreaks which were caused by S. Enteritidis were recorded with increasing frequency. 200000 cases were observed in 1992. The outbreaks were strongly associated with hen's eggs or chicken. Since 1985 we started to type S. Enteritidis with the phage typing system developed in East Europe (Laszlo) and later on with the international phage typing system according to Ward.
Currently, we use both the systems and describe the types separated by a diagonal strike. E.g. PT4/6 means PT4 according to the system of Ward and PT6 according to the system of Laszlo. Between 1984 and now approximately 50000 isolates of S. Enteritidis were phage typed. Additionally, approximately 2500 strains were typed by molecular methods (plasmid profile, PFGE, and others). As also shown by other authors macrorestriction fragment analysis by PFGE of PT4/6 strains no significant differences could be demonstrated. It was of evolutionary interest to see whether the strains caused the epidemic in 1972/73 (former GDR) were clonally different from 1984 to 1996. Reinvistigations of strains from 1973 show the same phage type 4/6 like the strains of 1995 but we found differences in macrorestriction fragment pattern by PFGE. This teach us that strains of 1973 did not survive. A new clone within the phage type 4/6 developed. On the other hand, phage types such as PT4/6 can occure in various clones due to the horizontal spread of distinct temperate phages.
Furthermore, some S. Enteritidis strains from rodents belonged to unusual phage types and carried not the 37 MDa plasmid. They have a 59 MDa plasmid. These strains differ also in their biochemical properties and were biotyped as S. Enteritidis variant danysz.

The complex typing of Salmonella strains with different methods support the epidemiological and ecological surveillance programme. Detection of reservoirs and transmission routes depend from isolation and complex typing. This knowledge should be use for prevention and control of Salmonellosis.

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