Review article

PLASMID PROFILE ANALYSIS OF SALMONELLA ENTERICA SEROTYPE ENTERITIDIS

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Plasmid profile analysis (PP) is a method of determining a number and size of plasmids in bacterial isolates. When using plasmid profile analysis in determining epidemiological strain for evaluation of this method, it is necessary to compare epidemiological strains with non-epidemiological ones isolated in the same period of time. Plasmid profile determination is performed by lysis of bacterial cell, chromosomal denaturation, sedimentation of cell fragments by centrifugation, and precipitation of DNA with ethanol. DNA is separated by gel electrophoresis based on its molecule mass. Plasmids could be also analyzed by restriction enzymes, as well as by direct techniques, such as hybridization and electronomicroscopically. Using of plasmid profile analysis enables resolving numerous outbreaks caused by *Enterobacteriaceae*, especially *Salmonella.* This method could be performed alone for *Salmonella enterica* serotype *Enteritidis*, but also as a complementary method to phage typing (phagotypization), and other molecular genetic based techniques. *Acta Medica Medianae 2008;47(2):54-57.*

Key words: *plasmid profile, Salmonella enterica serotype Enteritidis*

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Introduction

Since '70s of the past century, along with improving methods of plasmids isolation, the importance of researching techniques of total bacterial plasmids contents as a mean of determining clone concept rose up. Determination of a number and size of plasmids in bacterial isolates is called plasmid profile analysis (PP) (1,2,3,4). It appears that epidemical strains of Enterobacteriaceae, bacteria of Pseudomonas and Vibrio strains, staphylococcus and streptococcus can be identified in this way (4,5). However, isolates which are not connected with the outbreak may manifest epidemical profile. Thus, when using plasmid profile analysis in determining epidemical strain for evaluation of this method, it is necessary to compare epidemiological strains with non-epidemiological ones isolated in the same period of time (4).

Namely, determination of plasmids profile is performed after the growth of culture in a liquid or on a solid medium, by breaking off outer membrane of bacterial cell with lysosomes, inner membrane by detergent lysis, and chromosomes denaturation in alkali pH. Cell debris is sedimented by centrifugation in a microcentrifuge for a several minutes, after which DNA precipitation with ethanol follows. This method, modified to some degree, can be used for isolation of plasmid DNA from majority of genera and strains related to family of Enterobacteriaceae (4). Plasmid DNA is exposed to horizontal or vertical gel electrophoresis, while process of separation, based on molecule mass, occurs during migration toward anode. Additionally, gel is painted with ethidium bromide which binds to DNA and shows the fluorescent color when exposed to UV light.

Plasmid DNA with known molecule mass is recognized as a standard for every gel and its relative mobility is used in calculation of mass of unknown plasmids (4). As the length of migration relies on shape and dimension of plasmids, we can find more strips of plasmids on gel. Coiled molecules with covalent connections, which migrate at the highest speed, during extraction could be changed to open ring-shaped (circular) or linear forms. Since the coiled forms migrate faster than relaxed, their mutual relation and position in electrophoresis field are influenced by electric power, puffer system, the sizes of gel pores and DNA molecules. Therefore, appearing of several stripes could be interpreted in wrong manner as the existence of large number of

plasmids, instead of multiple forms of one single plasmid. On the other hand, as the electrophoresis mobility of two different plasmids could be the same, it is necessary to determine whether, based on examination of sequences of nucleotide bases, these plasmids have the same molecule masses and are genetically identical.

This analysis is performed by digestion of plasmid with restrictive endonucleases. Techniques used in plasmid analysis could be direct or indirect. The direct techniques include (i) DNA chains hybridization with calculation of homology degree (ii) direct electronomicroscopic heteroduplex analysis of small plasmids (nonhomological DNA would appear to be a simple loop), (iii) discovering of DNA sequences by Southern-blot hybridization with radioactive DNA probes. The indirect techniques are (i) agarose gel electrophoresis and (i) restriction endonuclease analysis (3).

Comparison of strains, based on their contents of plasmids or on restrictive profiles of plasmid DNA, is very useful in the case that a bacterium contains plasmids. However, when a bacterium does not contain plasmids, this method gives us very little benefit.

Plasmid profile analysis is useful in determining the epidemical strain in outbreaks caused by multiple species: Escherichia, Klebsiella, Pseudomonas, Serratia, Staphylococcus and so on (4). This method allowed successful identification of an epidemical strain P.aeruginosa isolated from patient on hemodialysis. Actually, the same strain was isolated both from patient flora and from iodoform solution which was associated with infection spread and removed from market afterwards (6).

Applying of plasmid profile analysis did confirm that usual humane infection was provoked by consumption of animal originated food. Identical resistance plasmids from Salmonella serovars isolated from humans and animals, in the case of Salmonella enterica serovar Typhimurium (S.Typhimurium) var Copenhagen, S. Newport, and S. Dublin have been obtained from different geographic areas in the US. Thanks to the plasmid profile analysis of these strains, it has been confirmed that they spread out by means of using animal-originated food. The method of restriction endonuclease analysis has shown that plasmids from animal and human isolates were quite often identical or almost identical (7).

In examination of epidemical strains S.Typhimurium there is a common opinion that method of plasmid profile analysis is easy to use, equally specific as phagotypization, and superior against biotypization and resistotypization (8). Plasmid profile analysis has enabled discovering of infection source associated with multiresistant (MR) strains of S.Typhimurim in Sao Paolo (Brazil). It is established that infections associated with strains with the same plasmid profile occurred among children hospitalized in the same hospital, thus endemic process consisted of series of small outbreaks raised by infection among children in some hospitals.

Plasmid profile analysis has proved as successful method in solving of outbreaks caused by S.Typhimurium in our laboratories, too. In an outbreak associated with this serovar occurred in a military base, Cobeljic et al. have detected epidemic strain in plasmids of 30 MDa and 60 MDa. The strain was isolated from the patient's stool, from the cook as a carrier of infection and from food. At the same time, analyzing the other outbreak in the base, the same authors pointed out that strains of the same serovar could be isolated from the outbreak, which were not associated with it (10).

Plasmid profile analysis enables solving outbreaks caused by other salmonellas. In an outbreak associated with S.Newport, in the US, it was established that a beef from the farm was the origin of epidemic since each strain, isolated from patients had R plasmid which conditioned resistance to ampicillin, carbencillin, and tetracycline. Though many people were infected, only those, who did allow selective advantage and growth by antibiotics, became ill (11).

In several US states, plasmid profile in strains isolated from patient's stools with enterocolitis caused by S. Muenchen, and from marihuana, allowed identification of an epidemic strain with unusual way of spreading (12). At the Health Care Institute in Novi Sad, there were isolates of S. Hadar, associated with an outbreak and obtained from patient's and the restaurant employees stools, and from food, resistant to ampicillin and streptomycin. Plasmid profile analysis revealed presence of five plasmid stripes with molecule masse of 13, 5.4, 4.2, 2.0, and 1.7 MDa (13).

By plasmid profile analysis, we can determine a different number of profiles, which depends on geographic location of isolates, time of researching, as well as the origin of strains, that is, whether they are isolated from human, animal or from food. In 2005, in Scotland, in 523 of 543 isolates (96.3%) of S. Enteritidis, which is one of the most common serovar, there was found one plasmid at least. In 269 of these isolates (49.5%), there was only virulence plasmid of 57 kb, and twenty isolates did not contain any plasmid. At 134 PT1 isolates there were described 16 different plasmid profiles, and 11 profiles were found in 117 PT4 isolates. In the rest of 292 isolates of S. Enteritidis, there were found 74 different plasmid profiles (14).

Investigating strains of S. Enteritidis isolated from people and different animals, there were identified sixteen different plasmid profiles which were used to make a difference between strains especially among most common phagotypes (PT 8 and PT 13a) (15). In the study carried out on 318 strains of S. Enteritidis isolated from poultry and its environment in Canada, there were evidenced 12 phagotypes. However, among these strains it was possible to determine fifteen different plasmid profiles (16).

Investigating plasmid profile of S. Enteritidis isolated from poultry during 1989 – '90 in Canada, the authors have proved twelve plasmid profiles against just four phagotypes

(17). Investigating 105 strains of S.Enteritidis (72 of them had human origin and 33 non-human origin) isolated in the period from 1975 to 1995, seven different plasmid profiles were proved, while 96% isolates had have plasmid of around 36 MDa (18). Investigating 64 isolates of S.Enteritidis from laboratory collection of Enterobacteriaceae at the University of Medical Science in Ankara, 88% of examined isolates had from 1 up to 4 plasmids which sizes varied from 2.5 to 100 kbp. Plasmid of 57 kbp was the most common and it was proved in 69% of isolates as a single one or along with the other plasmids (19).

Plasmid profile is determined for 276 strains of S.Enteritidis isolated at the Public Health Institute in Nis. From 94 isolates there were investigated 28 epidemic and 182 non-epidemic strains. Plasmid profile analysis of epidemic and non-epidemic strains showed up to 12 plasmid profiles. Among the two most common plasmid profile: PP 38 MDa and PP 38 2MDa, plasmid profiles with plasmid sizes of 4.0, 3.4, 2.7, and 1.5 MDa were determined too, as well as PP with large plasmids of 34 and 27Mda, though scattered (20).

Plasmid profile analysis was successfully applied to resolving outbreaks associated with S.Enteritidis. Researching the outbreak of enterocolitis at restaurant chain in Meriland, plasmid profile (55, 3.5, and 2.5 kb) was determined in isolates of S.Enteritidis isolated from every patient. The probability that this plasmid profile was randomly associated to the outbreak was less than 0.05 (21). By researching of an outbreak at the University of Medical Science in Ankara, it was determined that outbreak strains carried the same plasmid profile: three plasmids with size of 57, 40, and 3.0 kbp (19). An outbreak caused by S.Enteritidis phagotype 13 (S. Enteritidis PT13) occurred in Czech Republic. The strain belonged to rare phagotype and was susceptible to ampicillin. Both pre-epidemic and post-epidemic isolated strains were inspected by method of plasmid profile analysis. It was determined that ampicillin resistance was associated with plasmid of 200 kb, which was evidenced at S. Enteritidis (20).

Regarding PT 8 strains of S.Enteritidis, it is considered that spreading of this dominating clone with evidenced PP 38MDa mostly, did decrease discrimination ability of both typization systems. By investigation of 203 sporadic and epidemic isolates of S.Enteritidis isolated in Meriland between 1985 and 1990, ten plasmid profiles were identified. The most dominating profile was PP 55kba (38MDa); it was 86% of total plasmid profiles from isolates of this phagotype isolated from 1988 - '89 (23).

In another investigation, plasmid profile analysis was superior in comparison to phagotypization of PT 8 strains because it was possible to describe six different plasmid profiles versus single one phagotype. On the other hand, three strains without plasmid of 55 kba (38 MDa) could be differentiated by applying two additional phages (24). These results point out that, in some cases, determination of plasmid profile and phagotypization could be complementary methods.

By some other authors, determination of plasmid profile is an addition to phagotypization. Investigation of 27 phagotype representatives of S.Enteritidis revealed that only eleven plasmid profiles could be identified. Two profiles were proved in fifteen PT4 and 8 strains, which are two most common phagotypes in Great Britain. The plasmid profile variations useful for epidemiologic researches were found in thirteen phagotypes (25). S.Enteritidis PT 4 had nine different plasmid profiles (26,26). Thus plasmid profile analysis could be accepted as effective addition to phagotypization by opinion of mentioned authors. It also considered that plasmid profile analysis is very useful for differentiation between PT 4 strains (27).

Through another studies, the researches pointed out instability of S. Enteritidis' plasmids in isolates of different origin. Investigating a nosocomial outbreak associated with S. Enteritidis in US, partial differences between plasmid profiles among strains isolated from patients were determined (54 kb the first ones, 54, and 8.4 kb the second ones, and 54 and > 100 kb the third ones), food (54 kb) and chicken's ovary (54), while strain associated with fresh eggs did not contain any plasmids (28).

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ANALIZA PLAZMIDSKOG PROFILA SALMONELLAE ENTERICA SEROTIP ENTERITIDIS

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Analiza plazmidskog profila (PP) je određivanje broja i veličine plazmida u bakterijskim izolatima. Prilikom primene analize plazmidskog profila u određivanju epidemijskog soja za procenu ove metode, neophodno je porediti epidemijske sojeve sa neepidemijskim sojevima izolovanih u istom vremenskom periodu. Određivanje plazmidskog profila vrši se lizom bakterijske ćelije, denaturacijom hromozoma, taloženjem ćelijskih ostataka centrifugiranjem i precipitacijom DNK etanolom. DNK se razdvaja elektroforezom u gelu na osnovu molekulske mase. Plazmidi mogu biti analizirani i restrikcionim enzimima, ali i direktnim tehnikama, hibridizacijom i elektronomikroskopski. Primena analize plazmidskog profila je omogućila rešavanje brojnih epidemija izazvanih crevnim bakterijama, naročito salmonelama. Kada je u pitanju Salmonella enterica serotip Enteritidis, metoda se može koristiti sama, kao metoda komplementarna fagotipizaciji, ali se može dopunjavati i drugim molekularno-genetskim tehnikama. *Acta Medica Medianae 2008;47(2):54-57.*

Ključne reči: *plazmidski profil, Salmonella enterica serotip Enteritidis*