H. E. Müller

Listeria Isolations from Feces of Patients with Diarrhea and from Healthy Food Handlers

**Summary:** A study was undertaken on the presence and frequency of *Listeria* sp. in feces from 1,000 patients suffering from diarrheal diseases and from 2,000 healthy persons. Furthermore, the feces of patients were examined for other well-documented enteropathogens such as *Campylobacter*, *Salmonella*, *Shigella*, *Staphylococcus aureus*, *Yersinia enterocolitica*, protozoa and rotavirus as well as for organisms of questionable enteropathogenic potency such as fungi, i.e. *Candida*. Finally, in continuation of previously described investigations of the enteropathogenic role of *Proteus mirabilis* but not of *Proteus vulgaris*, both these species were studied too. Only *Listeria innocua* and *Listeria monocytogenes* could be detected in the investigated fecal specimens. There were no differences of the frequencies of *L. innocua*, and *L. monocytogenes* between patients and healthy persons. 17 strains (= 1.7%) of *L. innocua* and six strains (= 0.6%) of *L. monocytogenes* were isolated from 1,000 samples of patients. As a comparison 2,000 fecal samples from healthy people contained 40 strains (= 2.0%) of *L. innocua* and 16 strains (= 0.8%) of *L. monocytogenes*. A coincidence study showed that there were no statistically significant correlations between well-known enteropathogens and *Listeria* sp., *Proteus* sp. or any of the other isolates. Significant correlations were found only between harmless species such as *L. innocua* and *P. vulgaris*.

**Zusammenfassung:** Listerien im Stuhl von Diarrhöe-Patienten und von gesunden Personen aus dem Lebensmittelgewerbe. In der vorliegenden Studie wurde das Vorkommen und die Häufigkeit von Listerien in Stuhlproben von 1000 Patienten mit Diarrhöe und 2000 Gesunden untersucht, um das Spektrum der verschiedenen *Listeria*-Arten kennenzulernen. Zusätzlich wurden die Fäzes der Kranken auf bekannte darmpathogene Erreger wie *Campylobacter*, *Salmonella*, *Shigella*, *Staphylococcus aureus*, *Yersinia enterocolitica*, Protozoen und Rotaviren untersucht sowie auf Keime fraglich enteropathogener Bedeutung wie etwa *Hefepilze*. In Fortführung vorausgegangener Arbeiten wurden sowohl der enteropathogene *Proteus mirabilis* als auch der im Intestinaltrakt offensichtlich apathogene *Proteus vulgaris* in die Studie miteinbezogen. In den Fäzesproben konnten lediglich die beiden *Listeria*-Arten *Listeria innocua* und *Listeria monocytogenes* gefunden werden. Offensichtlich sind alle übrigen *Listeria*-Arten im menschlichen Darmtrakt wesentlich seltener. Die beiden vorhandenen kamen bei Gesunden und Kranken gleich häufig vor. Im Kollektiv der Gesunden konnten 40 *L. innocua*-Stämme (= 2.0%) und 16 *L. monocytogenes*-Stämme (= 0.8%) isoliert werden, bei dem Kollektiv der Diarrhöe-kranken waren es 17 *L. innocua*-Stämme (= 1.7%) und sechs *L. monocytogenes*-Stämme (= 0.6%). Daraus geht deutlich hervor, daß Listerien an der diarrhöischen Symptomatik unbeteiligt sind. Eine Koinzidenzstudie zur Frage möglicher Zusammenhänge zwischen verschiedenen enteropathogenen Erregern ergab keine statistisch signifikanten Korrelationen. Lediglich zwischen den beiden harmlosen Arten *L. innocua* und *Proteus vulgaris* wurde eine Korrelation gefunden.

**Introduction**

Listeriosis was first described in human beings more than 60 years ago [1]. However, its potential for common source of foodborne outbreaks as well as for single cases of intestinal disorder was not recognized until the last decade. During that time some documented epidemics drew attention to the organisms again [2–5].

Synchronously, our knowledge of listerial taxonomy increased and the original species *Listeria monocytogenes sensu lato* was subdivided into *L. monocytogenes sensu stricto* and several other apathogenic as well as pathogenic *Listeria* sp. [6, 7].

On the other hand the occurrence of *L. monocytogenes* in human fecal samples has been investigated since the early sixties [8, 9] by several authors. With the exception of one laboratory [10–13] all other studies found a frequency of about 5% *L. monocytogenes* in feces [2, 6, 7, 14].

The purpose of the present investigation was to evaluate the occurrence of different *Listeria* sp. in the feces of healthy persons on the one hand, and of patients suffering from diarrheal diseases on the other hand. Furthermore, the question of correlation between the occurrence of Listeria in stool samples and other known agents of infectious diarrhea was investigated.
Materials and Methods

Origin and collection of fecal specimens: 1,000 samples from patients suffering from diarrhea and 2,000 samples from healthy handlers serving food as controls were investigated. The fecal specimens were collected in stool preservations in the area of Braunschweig for six months (November 1988 – April 1989).

Methods of investigation:

**Listeria:** Stool samples were inoculated into nutrient broth no. 2 (Oxoid CM 67) and incubated at 4°C. After four weeks 1 ml was transferred to 10 ml of nutrient broth containing 37.5 g/l potassium thiocyanate and 100 mg/ml nalidixic acid (Sigma N 8878) and incubated at 36 +/- 1°C over night [15, 16]. Then the broth was streaked on Listeria selective medium (Oxford form, Oxoid CM 856 and SR 140) consisting of 39.0 g/l Columbia agar base, 1.0 g/l esculin, 0.5 g/l ammoniumferricitrate, and 15.0 g/l lithiumchloride supplemented with 400 mg/l cycloheximide, 20 mg/l colistinsulfonate, 5 mg/l acriflavine, and 10 mg/l fosfomycin, and incubated for two days at 30°C. The differentiation was performed biochemically according to standard procedures [7].

**Proteus:** Swarming strains of Proteus were isolated from blood agar after incubation at 36 +/- 1°C overnight and identified by biochemical standard procedures [17].

**Protozoa:** Examination for Blastocystis hominis, Cryptosporidium sp., Entamoeba histolytica, and Lambia intestinalis was performed by direct iodine wet mounts and the two stains Gomori's trichrome and Ziehl-Neelsen acid fast stain [18].

**Salmonella:** were enriched in both a selenite-cystine broth and in a tetrathionate brilliant green bile broth. The two broths were streaked on the two differentiation media xylose-lysine-deoxycholate and Wilson-Blair agar. Further differentiation was performed biochemically and serologically.

**Yersinia, Shigella, Staphylococcus, Yeasts:** Yersinia was isolated especially on Schiemann's CIN medium (Oxoid CM 653 and SR 109), Shigella on Endo- or MacConkey agar, Staphylococcus aureus on mannitol-salt agar (Oxoid CM 85) and yeasts on Sabouraud agar.

**Campylobacter:** The organisms were cultivated on a Preston Campylobacter blood medium (Virion) and incubated microaerobically in the CampyPac® system (BBL).

**Rotavirus:** Direct examination of stool specimens for rotavirus was performed by the ELISA procedure as described [18]. Rabbit immunoglobulin (B 218) and peroxidase-conjugated rabbit immunoglobulin (P 219) to human rotavirus were obtained from Dakopatts, Denmark.

Statistical methods: The Chi-square test was used for the statistical analyses.

Results

Altogether 79 strains of Listeria were isolated, i.e. 57 strains of *L. innocua* and 22 strains of *L. monocytogenes*. No other *Listeria* species could be found suggesting that they are generally present in an amount of less than 1–2%. Regarding the two groups of human beings there are no statistically significant differences between patients and healthy controls. The frequency of *L. innocua* as well as of *L. monocytogenes* expressed as a percentage is about the same as shown in Table 1.

The total number of enteropathogens identified in the group of patients is given in Table 2. The list contains not only known enteropathogens but also organisms which are partly absolutely harmless and partly of unclear pathogenic potency. Furthermore, Table 2 shows the occurrence of the listed organisms with each other. A strong correlation between *P. vulgaris* and *L. innocua* was evident while relatively weak correlations were found between *P. vulgaris* and *B. hominis* as well as between *Salmonella* and *Staphylococcus aureus* but no further correlations between others were evident.

Discussion

The results show clearly that *Listeria* are not enteropathogenic organisms. Nevertheless, they belong to the intestinal flora as transient or resident flora. Furthermore, we have to assume for that reason that *L. monocytogenes* invades from the intestine into the macroorganism where the organisms are able to exist intracellularly over some years. Surprisingly, the frequency of apathogenic *L. innocua* in human feces was much higher than that of pathogenic *L. monocytogenes* although waste water contains about ten times more *L. monocytogenes* than other Listeria species [16, 19]. However, our recent findings showed that *L. monocytogenes* ingested by protozoans survive and multiply intracellularly [20]. This observation may be an explanation for the excess of *L. monocytogenes* in waste water. *L. seeligeri*, also present in waste water, could not be detected in human feces indicating that their frequency is markedly less than that of *L. innocua* or *L. monocytogenes*. The coincidence of *L. innocua* and *P. vulgaris* in fecal specimens shown in Table 2 seems to have no consequences on the enteropathogenicity; also the weak correlation between *P. vulgaris* and *B. hominis* probably is of no real importance. They seem to have no disease-producing potential in the human intestine.

However, the study reinforces the findings of two previous publications dealing with *P. mirabilis* as an independent causative agent of intestinal disorders [21, 22] whereas *P. vulgaris* plays no pathogenic role. Of course, the strong correlation between *P. mirabilis* and *Y. enterocolitica* found

| Table 1: Listeria isolated from 1,000 fecal specimens of patients suffering from diarrheal diseases and from 2,000 fecal specimens from healthy people. |
|---------------------------------|-----------------|-----------------|-----------------|
| **Listeria**                   | **No. of strains isolated** |                  |
| Total                          | From 1,000 patients | From 2,000 healthy people |
| Listeria innocua               | 57 (= 1.9%)        | 17 (= 1.7%)      | 40 (= 2.0%)     |
| Listeria monocytogenes         | 22 (= 0.7%)        | 6 (= 0.6%)       | 16 (= 0.8%)     |
| Total                          | 79 (= 2.6%)        | 23 (= 2.3%)      | 56 (= 2.8%)     |