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Virulence potential of *Listeria monocytogenes* strains recovered from pigs in Spain

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Declarations of interest: none

Abstract

Background: *Listeria monocytogenes* is a foodborne bacterial pathogen that causes listeriosis, an infectious disease of animals and humans, with pigs acting as asymptomatic reservoirs. In August 2019 a listeriosis outbreak associated with the consumption of pork meat caused 222 human cases in Spain. Determining the diversity as well as the virulence potential of strains from pig origin is important for public health concerns.

Methods: The behavior of twenty-three *L. monocytogenes* strains recovered from pig tonsils, meat and skin was compared by studying: (1) Internalin A (InlA), Internalin B (InlB), Listeriolysin O (LLO), Actin assembly-inducing protein (ActA) and PrfA expression levels, and (2) invasion and intracellular growth in eukaryotic cells.

Results: Marked differences were found in the expression of the selected virulence factors and the invasion and intracellular replication phenotypes of the *L. monocytogenes* strains. Strains obtained from meat samples and belonging to ST 1/2a did not have InlA anchored to the peptidoglycan. Some strains expressed higher levels of the studied virulence factors and invaded and replicated intracellularly more efficiently than an epidemic *L. monocytogenes* reference strain (F2365).

Conclusion: This study demonstrates the presence of virulent *L. monocytogenes* strains in pigs, with valuable implications in veterinary medicine and food safety.

Keywords: isolates; invasion; replication; virulence factor; tonsil; meat.

Introduction

Listeria monocytogenes is a Gram-positive facultative intracellular pathogen responsible for listeriosis, a food-borne zoonotic disease of animals and humans that causes gastroenteritis, meningoencephalitis and abortions, with a high mortality rate. *L. monocytogenes* has been isolated worldwide from humans, animals, environmental sources and food sources like meat. Food-producing animals are significant reservoirs in disseminating this bacterium. Although all *L. monocytogenes* strains are currently treated equally for regulatory purposes, some strains are highly virulent and are more often associated with epidemics, while others are less virulent and are infrequently related to epidemics ⁽¹⁻³⁾. Particularly, in human listeriosis outbreaks, isolates belonging to the serotype (ST) 4b are overrepresented, with a lower number of cases associated with isolates from the ST 1/2a which is usually associated with food and food-related *L. monocytogenes* isolates ^(4, 5).

In 2018, 28 countries reported 2,549 confirmed listeriosis cases in the EU/EEA with more than 229 associated deaths ⁽⁶⁾. In 2017-2018 a listeriosis outbreak related to ready-to-eat processed meat products in South-Africa affected 1,060 human patients, of who 216 died ⁽⁷⁾. More recently, in August 2019 the Regional Health Authorities in Andalusia, Spain, reported a listeriosis outbreak associated with the consumption of pork meat which caused 222 confirmed cases with 3 deaths, 6 miscarriages and one travel-related case in France ⁽⁸⁾. Therefore, from a public health point of view, it is essential to evaluate the virulence potential of strains that colonize food-producing animals, since differences in virulence between *L. monocytogenes* strains may influence infection and clinical outcome ^(1, 9).

L. monocytogenes possess several virulence factors which, after ingestion of contaminated food, play a significant role in the cellular internalisation, vacuolar escape, and cell-to-cell spread. The major bacterial virulence factors for the successive steps of the cell infectious process are the surface proteins Internalin (Inl) A (InlA) and InlB for the internalisation process, the pore-forming toxin Listeriolysin O (LLO) for the escape from the vacuole and the Actin assembly-inducing protein (ActA) for the actin-based intracellular motility. PrfA is the major transcriptional regulator of virulence genes (reviewed in ⁽⁵⁾). Importantly, during the last decades, the majority of *L. monocytogenes* studies in bacterial pathogenesis compared three major laboratory strains: EGD, EGD-e, and 10403S, all of them belonging to the ST 1/2a ⁽¹⁰⁻¹⁴⁾. Major listeriosis epidemics have been preferentially associated with *L. monocytogenes* of ST 4b ^(4, 15). Moreover, concerns about the adequacy of laboratory-adapted reference strains for the study of field isolates pathogenesis have risen from 1) the plasticity of bacterial genomes which enables bacteria to rapidly adapt to *in vitro* conditions; and 2) the continuous sub-culturing of bacteria which may lead to the loss of important pathogenic characteristics ⁽¹⁶⁾.

L. monocytogenes is a ubiquitous bacterium that has been previously isolated along the pork production chain either from environmental or animal samples ^(17, 18). The pork production sector has been hit hard by a series of *L. monocytogenes*-related food poisoning outbreaks, and many studies have determined the serotypes or PCR serogroups most commonly found in pig slaughterhouses ^(19, 20). However, no studies are evaluating the potential of *L. monocytogenes* isolates from pig origin to express virulence factors, invade and grow intracellularly in human cells. We thus decided to study the variability in the expression of InlA, InlB, LLO, ActA and

PrfA in twenty-three *L. monocytogenes* strains of different ST (1/2a and 4b) recovered from pig tonsils, meat and skin in Spain as well as their phenotype during human host cell infections.

Material and methods

Ethics approval statement

This experiment and all animal procedures were performed according to the guidelines of the European Union (Directive 2010/63/EU). As the data used in this study were part of the routine slaughterhouse activity no ethical committee approval was needed.

Bacterial strains and eukaryotic cell lines

The *L. monocytogenes* strains from pigs used in this study were selected from a previous study⁽²¹⁾. The origin of the bacterial isolates and their ST are listed in Table 1. The *L. monocytogenes* strain F2365 (ST 4b) responsible for the 1985 California listeriosis outbreak associated to contaminated cheese and characterised in recent studies^(11, 12, 22, 23) was used as a reference strain⁽²⁴⁾. The *L. monocytogenes* strains were grown overnight in brain heart infusion (BHI). The epithelial cell line JEG-3 (ATCC HTB-36), commonly used in *L. monocytogenes* studies^(12, 25) was propagated in Dulbecco's modified Eagle medium (DMEM) supplemented with 10 % foetal bovine serum (FBS).

Western blot analysis

Bacteria grown overnight in BHI to stationary phase in shaking conditions at 37 °C were pelleted and re-dissolved in 1 ml of PBS and complete protease inhibitor and lysed by sonication. For cell wall protein analysis, subcellular fractions containing protoplasts and peptidoglycan associated

proteins were obtained by mutanolysin treatment as previously described^(25, 26). Extracts were adjusted to load the same number of bacteria of all strains. Load control was performed using the BioRad stain-free imaging technology (proteins are made fluorescent directly in the gel with a short photoactivation, allowing the immediate visualisation of proteins). A volume of protoplasts (5µl) and the cell wall fraction (2µl) was analysed by SDS-PAGE followed by Western blot using standard protocols for membrane blocking and antibody incubation⁽²⁷⁾. The following primary antibodies were used: mouse monoclonal anti-InlA (dilution 1:1000)⁽²⁸⁾, rabbit polyclonal anti-InlB R25 (dilution 1:10000)⁽²⁷⁾, rabbit polyclonal anti-LLO R176 (dilution 1:20000)⁽²⁹⁾, rabbit polyclonal anti-ActA R32 (dilution 1:1000)⁽³⁰⁾, and rabbit polyclonal antibody raised against PrfA R78 (dilution 1:1000) (Covalab UK Ltd, United Kingdom). HRP-conjugated antibodies (AbCys S.A., France) were used as secondary antibodies (dilution 1:8000). Proteins were revealed using Pierce ECL 2 Western Blotting Substrate (Thermo Fisher, USA).

Spectrophotometric growth analysis

The kinetics of *L. monocytogenes* strains grown in BHI was recorded using an automated 96-well plate reader (Sunrise Tecan, Switzerland) to measure OD₆₀₀.

Infection of eukaryotic cell lines and determination of invasion and proliferation rates

Cell infections were performed by using gentamicin protection assays as previously described⁽²³⁾. Briefly, JEG-3 cell suspensions were seeded in 96-well tissue culture plates and grown for 24 h in an antibiotic-free medium. On the day of infection, cells were then incubated with the bacteria at a multiplicity of infection of 5 for 1 h at 37°C. Following this incubation, the cells

were washed, and extracellular bacteria were neutralized by adding complete medium containing 40 µg/ml of gentamicin. At 1 h 30 min or 5 h post-infection, cells were washed with PBS and finally lysed and the number of viable intracellular *L. monocytogenes* was calculated. *In vitro* invasion rates were calculated as the number of viable intracellular bacteria counted at 1 h 30 min post-infection. Intracellular replication was determined as the number of viable intracellular bacteria counted at 5 h post-infection (hpi). The index of proliferation was calculated by dividing the number of viable intracellular bacteria at 5 h by the number of viable intracellular bacteria obtained at 1 h 30 min. These experiments employed 6 technical replicates per bacterial strain and were repeated three times with independent clones of each of the strains.

Statistical analysis

The values were evaluated for approximate normality of distribution by the D'Agostino & Pearson omnibus normality test (GraphPad Prism v7.0, USA). Since data regarding growth kinetics, invasion and proliferation rates of *L. monocytogenes* strains followed a normal distribution, comparison between groups was assessed by ANOVA test followed by Fisher's LSD test (GraphPad Prism v8.0, USA). Differences with a $P < 0.05$ were considered to be statistically significant.

Results

InlA, InlB, LLO, ActA and PrfA expression levels in *L. monocytogenes* strains

Twenty-three *L. monocytogenes* strains isolated from pigs of ST 1/2a and 4b were selected for characterisation of the expression of major virulence factors based on their origin, sample source and serotype⁽²¹⁾. The *L. monocytogenes* strain F2365 (ST 4b) associated with a Mexican-style

cheese listeriosis outbreak (142 human cases, 48 deaths) was included as an epidemic reference strain for comparison^(11, 12, 22-24). Marked differences were found at the protein level for InlA, InlB, LLO and ActA among the strains (Figure 1 and Supplementary Figure 1). Conversely, PrfA levels were quite homogenous across *L. monocytogenes* strains, suggesting that the differences in the expression level of the analysed virulence factors are not due to differential amounts of this transcriptional regulator (Figure 1 and Supplementary Figure 1). No pattern in terms of InlA, InlB, LLO, ActA or PrfA expression profiles could be found to differentiate *L. monocytogenes* 1/2a and 4b strains. Interestingly, three strains (G6C6, G8C13, G10C14) obtained from meat samples and belonging to ST 1/2a did not have InlA anchored to the peptidoglycan (Figure 1 and Supplementary Figure 1). However, this finding was not observed in other ST 1/2a strains. As previously reported, InlB was not detected in *L. monocytogenes* F2365 due to a nonsense mutation in *inlB*^(12, 31) (Figure 1 and Supplementary Figure 1). According to our findings, no clear correlation could be found between the protein levels of the virulence factors analysed: for example, the strains G3T6 and G3T7 displayed high levels of LLO and InlA whilst the strains G8T7 and G10T1 presented high levels of LLO but low levels of InlA. Another example is given by the strain G8T4 which displays low levels of LLO and ActA and the strain G3T7 which showed high levels of LLO but low levels of ActA. The distinctive InlA, InlB, LLO, ActA and PrfA profiles of each *L. monocytogenes* strain are summarised in Table 1.

Invasion and intracellular replication of *L. monocytogenes* strains in eukaryotic cells

All the *L. monocytogenes* strains from this study demonstrated similar growth characteristics in BHI (Figure 2A). The strain F2365 grew slower than other *L. monocytogenes* strains as previously reported (24) (Figure 2A).

To evaluate the ability of the *L. monocytogenes* strains to invade and replicate within eukaryotic cells, gentamicin assays in JEG-3 cells were performed. Eight different *L. monocytogenes* strains (4 strains of ST 1/2a and 4 strains of ST 4b) were selected based on their distinctive InlA, InlB, LLO and ActA profiles (Table 1). The strains G5T14 (ST 1/2a), G8T7 (ST 4b), G10T1 (ST4b) and G10T14 (ST 1/2a) were significantly more invasive than the F2365 strain (ST 4b) ($p<0.05$) (Figure 2B). Accordingly to the absence of InlA in the cell wall, the strains G6C6 (ST 1/2a) and G10C14 (ST 1/2a) were less invasive than the rest of strains and the F2365 strain ($p<0.05$) (Figure 2B). Regarding intracellular proliferation, the strains G3T6 (ST 4b), G3T7 (ST 4b), G5T14 (ST 1/2a), G6C6 (ST 1/2a) and G10C14 (ST 1/2a) replicated more efficiently than the epidemic F2365 strain (ST 4b) ($p<0.05$) (Figure 2C and 2D). Although the strains G6C6 and G10C14 entered less efficiently in JEG-3 cells due to the absence of InlA in the cell wall, their index of proliferation within cells was similar to other strains demonstrating the normal activity of the virulence factors involved in the intracellular lifestyle (e.g. LLO and ActA) (Figure 2B-2D). No specific pattern in terms of invasion or intracellular replication profiles could be found to differentiate *L. monocytogenes* 1/2a and 4b strains; however, as mentioned above, *L. monocytogenes* strains isolated from meat samples were less invasive but proliferated equally (or more) than those coming from other sampling sources. Altogether, these results show the existence in pigs of a diverse population of *L. monocytogenes* strains in terms of eukaryotic cell invasion and intracellular replication abilities, revealing the presence of strains (e.g. G5T14) that

invade and replicate intracellularly more efficiently than a *L. monocytogenes* strain that was able to cause an outbreak (F2365).

Discussion

L. monocytogenes strains present in pigs can be transferred to food processing environments, and thus contaminate food and it is, therefore, important to evaluate the virulence of these strains. Importantly, not all *L. monocytogenes* strains are equally virulent, some strains are highly virulent and are more often associated with epidemics, while others are less virulent and are infrequently related to epidemics ⁽¹⁻³⁾. Previous studies have shown that Lineage I (or serotype 4b) occurs more frequently among clinical isolates than lineage II (or serotypes 1/2b, 1/2a and 1/2c), relative to the frequency of these categories in food ⁽⁴⁾. Besides intrinsic *L. monocytogenes* virulence other extrinsic factors play equally an important role in listeriosis outbreaks, such as individual susceptibility, food category, good hygiene practices and good manufacturing practices, and persistence into the environment or biofilm formation among others ⁽³²⁾. Understanding virulence aspects of *L. monocytogenes* in animals and food supply chains can help regulators and operators to design more effective microbial surveillance and prevention strategies ⁽¹⁾. In this sense, more strict control measures, such as lower limit of bacterial load in food could be implemented when especially virulent *L. monocytogenes* strains are identified. In the present study, we addressed the issue of the general virulence potential of *L. monocytogenes* strains from pig samples in Spain.

Major foodborne listeriosis epidemics have been preferentially associated with *L. monocytogenes* of ST 4b ^(4, 15). However, during the last decades, the majority of *L. monocytogenes* studies in

host-pathogen interactions have compared three major laboratory strains: EGD, EGD-e, and 10403S all of them belonging to the same ST (1/2a)⁽¹⁰⁻¹⁴⁾. Besides, the occurrence of different strains of *L. monocytogenes* in reported cases of listeriosis together with the recovery of diverse isolates from the food industry environment^(18, 21, 33) highlights the necessity of evaluating the phenotypic diversity of *L. monocytogenes* isolates from food-producing animals. Here, using molecular and cell biology techniques, a high diversity was found regarding the expression of *L. monocytogenes* virulence factors in isolates from pigs in Spain. No specific pattern in terms of virulence factor expression, invasion or intracellular replication profiles could be observed in our study to distinguish *L. monocytogenes* 1/2a and 4b food isolates.

The strains G6C6, G8C13, G10C14 obtained from pig meat samples did not have InlA anchored to the peptidoglycan and consequently were less invasive than the rest of strains and the F2365 strain (Figure 1, Figure 2B and Supplementary Figure 1). Several mutations causing premature stop codons have been reported for the gene *inlA*^(34, 35). These premature stop codons cause loss of anchorage of InlA to the peptidoglycan and invasion attenuation⁽³⁶⁾. Premature stop codons in the *inlA* gene are more frequent in food isolates ($\approx 30\%$) than in human clinical isolates ($< 2\%$)⁽³⁷⁻³⁹⁾. Accordingly, in our study, the strains without InlA anchored to the cell wall were those strains isolated from meat samples (G6C6, G8C13, G10C14) but not those strains from the tonsils or the skin.

Although internalisation has been previously associated to be mediated by InlA, InlB, LLO and ActA^(5, 25, 40, 41), here we have demonstrated that the strain G3T6 possessing an InlA^{high}InlB^{high}LLO^{high}ActA^{med}PrfA^{high} profile invaded less efficiently than the G5T14

248 (InlA^{med}InlB^{high}LLO^{low}ActA^{low}PrfA^{low}), G8T7 (InlA^{low}InlB^{high}LLO^{high}ActA^{med}PrfA^{med}), G10T1
249 (InlA^{low}InlB^{high}LLO^{high}ActA^{high}PrfA^{med}) and G10T14 (InlA^{low}InlB^{high}LLO^{med}ActA^{high}PrfA^{med})
250 strains. These results suggest that invasion could be also mediated by the activity of additional
251 virulence factors (i.e. the invasins LPXTG surface protein Lmo1413)⁽³⁵⁾ and the interaction of all
252 of these factors simultaneously. Recent studies regarding bacterial population genomics have
253 harnessed the biodiversity of *L. monocytogenes* and uncovered multiple new putative virulence
254 factors^(4, 42). These discoveries will be helpful for further pathogenesis, diagnostic, phylogenetic
255 and functional research studies.

256
257 Regarding intracellular proliferation, we showed that the intracellular CFU numbers of the
258 epidemic strain F2365 at 5 hpi were lower than for other pig strains which suggest that one or
259 more of the 20 mutations resulting in premature stop codons of this F2365 strain could play a
260 fundamental role in its intracellular lifestyle⁽³¹⁾. Interestingly, the strain G8T7 which expresses
261 high levels of LLO and moderate levels of ActA proliferated less intracellularly than the strain
262 G5T14 which showed low protein levels of LLO and ActA. These results point out that, as
263 previously described for the invasion process, more virulence factors besides the classical ones
264 (i.e. LLO and ActA) could play an important role during intracellular proliferation. Even though
265 the meat pool strains G6C6 and G10C14 invaded eukaryotic cells less efficiently due to the lack
266 of InlA in the cell wall, they proliferated intracellularly similar to other strains used in the
267 present study or even more than the epidemic strain F2365. This is important because: 1) a *L.*
268 *monocytogenes* clinical isolate expressing a truncated form of InlA was responsible for a case of
269 septicemia⁽³⁹⁾; and 2) the strain F2365 responsible for the 1985 California listeriosis outbreak

also lacked one of the main virulence factors involved in entry (due to a nonsense mutation in *inlB*), but caused one of the largest epidemics of listeriosis (142 human cases, 48 deaths) ^(22, 24).

Other studies have also compared the ability of *L. monocytogenes* strains from different origins (human isolate, cow faeces, ground pork and cheese; and frozen whole eggs, cheeses, egg, ice cream, milk and frozen yolk, respectively) to invade cells, revealing widely different levels of invasion among the tested strains ⁽⁴³⁾. However, other studies that analysed *L. monocytogenes* strains from sausages, fish processing plants and a human clinical case ⁽⁹⁾ did not find differences among strains' ability to proliferate intracellularly, since all the tested *L. monocytogenes* strains presented identical intracellular growth rates in Caco-2 cells. It is now apparent that the restricted use of the most frequently used laboratory *L. monocytogenes* strains has masked the existence of this high diversity of isolates.

The virulence potential of *L. monocytogenes* strains recovered from pigs and their related food products is unknown. As far as we know, this is the first report documenting the virulence characteristics of *L. monocytogenes* recovered from pigs in Spain. Our analysis of twenty-three *L. monocytogenes* strains recovered from pig tonsils, meat and skin establishes the existence of a diverse population of *L. monocytogenes* in terms of virulence factors expression, eukaryotic cell invasion, and intracellular replication abilities. No specific pattern in terms of virulence factor expression, invasion or intracellular replication profiles could be found to differentiate *L. monocytogenes* 1/2a and 4b strains or from different sources, beyond the lower invasion for strains isolated from meat samples. Moreover, these results show the existence of some strains from pigs that invade and replicate intracellularly more efficiently than an epidemic *L.*

monocytogenes strain (F2365). These results have valuable implications in terms of veterinary medicine and food safety, since they highlight the importance of determining *L. monocytogenes* virulence to accordingly implement rigorous control measures and point out the necessity of identifying all the virulence factors involved in *L. monocytogenes* pathogenesis.

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The authors have no conflict of interest to declare.

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Figure legends

Figure 1. InlA, InlB, LLO, ActA and PrfA protein levels detected by Western blot. Bacteria were grown in BHI to stationary phase in shaking conditions at 37 °C. Extracts were adjusted to load the same number of bacteria of all isolates. Load control was performed using the BioRad stain-free imaging technology (proteins are made fluorescent directly in the gel with a short photoactivation, allowing the immediate visualisation of proteins)

Figure 2. *L. monocytogenes* isolates growth in laboratory medium and invasion and intracellular replication in eukaryotic cells. (A) Bacteria from overnight BHI culture were collected, washed, and added to fresh BHI medium. The OD was monitored at the indicated sampling time points (hours). Averages of three independent experiments were plotted, with error bars denoting standard deviation. (B & C) Numbers of viable intracellular *L. monocytogenes* isolates and F2365 strain cells in JEG-3 cells. CFU numbers were monitored at 1 h 30 min (B) and 5 h (C) p.i. (washed after 30 min of infection with 40 µg/ml gentamicin added). (D) Index of intracellular proliferation calculated by dividing the number of viable intracellular bacteria at 5 h by the number of viable intracellular bacteria obtained at 1 h 30 min. The standard deviation is shown. Three independent experiments with 6 replicates in each experiment were performed. One representative experiment is shown. Bars showing different letters represent values significantly different from each other ($p < 0.05$).

Supplementary material

Supplementary Figure 1: InlA, InlB and ActA protein levels detected by Western blot. Bacteria were grown in BHI to stationary phase in shaking conditions at 37 °C. Extracts were adjusted to

451 load the same number of bacteria of all isolates. Load control was performed using the BioRad
452 stain-free imaging technology (proteins are made fluorescent directly in the gel with a short
453 photoactivation, allowing the immediate visualisation of proteins). The InlA, InlB and ActA
454 panels in this figure are the same as in figure 1 but highly exposed.

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Table 1. *L. monocytogenes* isolates origin (21), serotype and distinctive InlA, InlB, LLO, ActA, and PrfA profiles.

Farm	Lab code	Serotype	Source	Virulence factor Profile
#3	G3T6	4b	Tonsils	InlA ^{high} InlB ^{high} LLO ^{high} ActA ^{med} PrfA ^{high}
#3	G3T7	4b	Tonsils	InlA ^{high} InlB ^{low} LLO ^{high} ActA ^{low} PrfA ^{med}
#3	G3T8	4b	Tonsils	InlA ^{low} InlB ^{low} LLO ^{high} ActA ^{low} PrfA ^{med}
#4	G4T2	1/2a	Tonsils	InlA ^{high} InlB ^{high} LLO ^{med} ActA ^{low} PrfA ^{low}
#4	G4T5	1/2a	Tonsils	InlA ^{high} InlB ^{low} LLO ^{low} ActA ^{low} PrfA ^{low}
#4	G4T6	1/2a	Tonsils	InlA ^{high} InlB ^{med} LLO ^{low} ActA ^{med} PrfA ^{med}
#5	G5T14	1/2a	Tonsils	InlA ^{med} InlB ^{high} LLO ^{low} ActA ^{low} PrfA ^{low}
#6	G6C6	1/2a	Meat pool	InlA ^{neg} InlB ^{high} LLO ^{high} ActA ^{med} PrfA ^{high}
#7	G7T1	4b	Tonsils	InlA ^{med} InlB ^{low} LLO ^{low} ActA ^{low} PrfA ^{low}
#7	G7T6	4b	Tonsils	InlA ^{high} InlB ^{low} LLO ^{low} ActA ^{low} PrfA ^{low}
#8	G8T4	4b	Tonsils	InlA ^{low} InlB ^{med} LLO ^{low} ActA ^{med} PrfA ^{med}
#8	G8T7	4b	Tonsils	InlA ^{low} InlB ^{high} LLO ^{high} ActA ^{med} PrfA ^{med}
#8	G8T14	4b	Tonsils	InlA ^{high} InlB ^{low} LLO ^{high} ActA ^{med} PrfA ^{med}
#8	G8E6	4b	Skin abrasive sponge	InlA ^{low} InlB ^{low} LLO ^{high} ActA ^{low} PrfA ^{high}
#8	G8C13	1/2a	Meat pool	InlA ^{neg} InlB ^{high} LLO ^{med} ActA ^{med} PrfA ^{med}
#9	G9T6	4b	Tonsils	InlA ^{med} InlB ^{low} LLO ^{med} ActA ^{low} PrfA ^{low}
#9	G9T10	4b	Tonsils	InlA ^{med} InlB ^{low} LLO ^{med} ActA ^{med} PrfA ^{med}
#9	G9T12	4b	Tonsils	InlA ^{high} InlB ^{low} LLO ^{low} ActA ^{med} PrfA ^{high}
#10	G10T1	4b	Tonsils	InlA ^{low} InlB ^{high} LLO ^{high} ActA ^{high} PrfA ^{med}
#10	G10T4	4b	Tonsils	InlA ^{low} InlB ^{med} LLO ^{med} ActA ^{med} PrfA ^{med}
#10	G10T5	4b	Tonsils	InlA ^{low} InlB ^{low} LLO ^{high} ActA ^{med} PrfA ^{high}
#10	G10T14	1/2a	Tonsils	InlA ^{low} InlB ^{high} LLO ^{med} ActA ^{high} PrfA ^{med}
#10	G10C14	1/2a	Meat pool	InlA ^{neg} InlB ^{high} LLO ^{med} ActA ^{low} PrfA ^{med}

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