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Six Years of *Pasteurella* spp. in the Pitiusas

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Abstract: *Pasteurell*a spp. is a gram-negative bacterium that is part of the oral and upper respiratory tract microbiota of many animals such as canids and felines. In humans it can produce pathology primarily associated with animal bites or scratches. In addition, members of this genus, and especially *Pasteurella multocida*, may also be involved in systemic infections, mainly in immunocompromised patients in close contact with pets. The aim of this study is to analyse the prevalence and clinical characteristics of infections caused by *Pasteurella* spp. as well as the sensitivity profile of these isolates obtained in the Microbiology laboratory of the Hospital Can Misses (Ibiza, Ibiza and Formentera Health Area, ASEF) in the period from January 2013 to December 2018. The group of islands of Ibiza and Formentera is known as the Pitiusas Islands. Retrospective descriptive study in which all *Pasteurella* spp. isolates were analysed in the Microbiology Laboratory of Hospital Can Misses from 1 January 2013 to 31 December 2018. Bacterial identification was performed by Vitek 2 automated biochemical test panel (bioMérieux, Spain) and antimicrobial susceptibility by E-test or antibiotic discs in Mueller-Hinton blood agar, interpreted based on European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards, 2022 version. Medical records were systematically reviewed by collecting demographic data of infected patients, comorbidities, epidemiological data and clinical features of the infection. A total of 22 isolates of *Pasterurella* spp. were obtained from 22 different patients, 62.8% female, from three different species: 18 *P. multocida*, 2 *P. canis* and 2 *P. pneumotropica*. Most isolates came from soft tissue infection samples: 7 wound exudates and 5 abscess material. The antibiotics tested with the highest sensitivity profile were gentamicin and cefepime (100% sensitivity). The species most commonly isolated in our environment is *P. multocida*. Close contact with companion animals, especially cats and dogs, is the main risk factor for *Pasteurella* spp. infections, although in many cases such contact cannot be established. So *Pasteurella* spp. infection should be considered a zoonosis in possible emergency, especially in rural areas such as Pitiusas Islands.

Keywords: *Pasteurell*a spp; pets; *P. multocida*; beta-lactams; skin and soft tissue infection; urinary tract infection

1. Introduction

Different species of *Pasteurella* spp. are among the major human pathogens originating from both wild and domestic animal wildlife, with a special focus on dogs and cats (1). The most common way of acquiring a *Pasteurella* spp. infection is through direct contact following a bite or scratch by a dog or cat, or also through licking open wounds. Many *Pasteurella* species can be pathogenic in humans, such as *P. dogmatis*, *P. canis*, *P. stomatis*, etc., but the most frequently isolated species is *P. multocida*, which has several subspecies: *multocida, septica* or *gallicida*. The most frequent of the subspecies in human pathology is *P. multocida* sbs *multocida* and is associated as an anthropozoonosis in many warm-blooded animals (2). *P. multocida* subsp. *septica* is associated with cat wounds and *P. multocida* subsp. *gallicida* is rarely found in human isolates.

There are multiple factors that cause this facultative anaerobic gram-negative coccobacillus, *Pasteurella* spp. to survive in the host: the polysaccharide membrane, the capsule and its resistance to phagocytosis, adhesive properties, various enzymes that act on the extracellular matrix facilitating dissemination and often the production of *P. multocida toxin* (PMT) (2). Some authors associate *Pasteurella* spp. bacteremia with comorbidities such as cancer (3).

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Pasteurella spp. causes in most cases localised infections such as cellulitis, abscesses, or arthritis near the site of inoculation, but can also lead to bacteremia (4), endocarditis or deep organ infections by hematogenous spread (2). Contact with cats seems to be more associated with bacteremia and contact with dogs, especially if a bite occurs, with local and superficial infections (5).

Understanding the full spectrum of *Pasteurella* spp. infections allows for a balanced perspective on the severity of such infections, risk of complications and most appropriate treatment. This study reviews the demographic, clinical and microbiological characteristics of patients in whom *Pasteurella* spp. was isolated at least once in the Microbiology laboratory covering the entire area of Ibiza and Formentera (Pitiusas Islands) over a period of six years.

2. Results

A total of 22 patients were included (mean age 65.9 years; only one minor, an 11-day-old neonate); 7 males (31.8%) and 15 females (68.2%). Isolations were 18 *P. multocida*, 2 *P. canis* and 2 *P. pneumotropica*. Cultures requested were as follows: 7 wound exudate cultures, 5 cultures of abscess material, 3 blood and 3 urine cultures, 2 joint fluid cultures and finally 1 sputum and 1 stool culture. 20 of the requests came from hospital EDs (90.9%) and 2 from primary care (9.1%). On 8 occasions the patient had contact with domestic animals that had caused an injury and in all 8 cases the micro-organism isolated was *P. multocida*; 7 were dog bites and 1 was a cat scratch. *P. pneumotropica* was isolated in a stool culture and in a urine culture with a significant count, and *P. canis* in an abscess and in a urine culture with a significant count as well. The 3 blood cultures with isolation of *Pasteurella* spp. were requested for urinary tract infection (UTI), cellulitis and fever of unknown origin. *Pasteurella* spp isolates over the years were 5 in 2013, 4 in 2014, 4 in 2015, 3 in 2016, 2 in 2017 and finally 2 in 2018. The sensitivity to the different antibiotics is reflected in Table 1.

Table 1. Total susceptibility of *Pasteurella* spp. isolates.

	Resistant	Intermediate / Susceptible with increased exposure	Susceptible (%)	Not tested
Ampicillin	1	2	18 (81.8%)	1
Amoxicillin/Clavulanic acid	1	1	20 (90.9%)	0
Cefuroxime	0	0	21(95.5%)	1
Cefotaxime	0	0	21(95.5%)	1
Ceftazidime	0	2	19 (86.4%)	1
Cefepime	0	0	22 (100%)	0
Imipenem	0	0	21(95.5%)	1
Amikacin	0	0	21(95.5%)	1
Gentamicin	0	0	22 (100%)	0
Ciprofloxacin	0	0	21(95.5%)	1
Trimethoprim - Sulfomethoxazole	2	0	19 (86.4%)	1
Piperacillin / Tazobactam	0	0	18 (81.8%)	8
Fosfomycin	1	0	2	19

*Antibiotic susceptibility interpreted according to EUCAST 2022 criteria.

3. Discussion

The genus *Pasteurella* was named after Luois Pasteur, who was the first to isolate this bacterium in 1880 as the causative agent of fowl cholera. *Pasteurella* ssp. and especially *P. multocida*, is one of the most frequent commensal microorganisms found in domestic animals worldwide, being very common in the oropharynx of dogs and cats, and most infections in humans are related to exposure to these pets, as in our study with 8 cases of *P. multocida* isolation with direct inoculation by cat or dog wounds (6). Cats are the animals with the highest percentage of colonisation by *Pasteurella* spp.

with 70-90%, followed by dogs with 20-50%. Other microorganisms frequently isolated from animal bite or scratch wounds are *Bartonella henselae* or *Staphylococcus aureus*.

This is a small, gram-negative, facultatively anaerobic, immobile, oxidase- and catalase-positive, gram-negative coccobacillus, which typically stains bipolar with methylene blue (7). *Pasteurella* spp. grows on blood agar and chocolate agar, but not on MacConkey agar. It grows well on TSA (Trypticase Soy Agar) or BHI (Brain-Heart Agar) enriched media. Transmission occurs even through small microtrauma caused mainly by cats and dogs. Once inoculated in humans, it can produce a wide spectrum of infection, both local and invasive, with particular attention to cellulitis and osteomyelitis at sites close to the point of inoculation (8)(9). Despite acting as an opportunistic organism, *P. multocida* has a high pathogenic potential due to various virulence factors such as capsular lipopolysaccharide, cytotoxin, haemagglutinin, adhesins and iron sequestering proteins (2). Therefore, complications of local infection such as necrotising fasciitis, bacterial arthritis, endocarditis, meningitis, and sepsis are not uncommon after trauma.

In the existing literature, the most isolated species in human pathology are *P. multocida*, especially the subspecies multocida, *P. canis* and *P. dogmatis*, the latter being absent in our cases, where we did find *P. pneumotropica* (5). There are 5 serogroups of *P. multocida*, A, B, D, E and F, with A and D being the most isolated in human pathology, especially associated with animal oral microbiota (7). The most frequent of the subspecies in human pathology is *P. multocida multocida* (2). *P. multocida septica* is usually found in cat scratch wound infections and *P. multocida* subsp. *gallicida* rarely causes pathology in humans.

Both in the literature and in our experience, we found a wide spectrum of infections caused by *Pasteurella* spp. but skin and soft tissue infections after inculcation of the organism by violent contact with the animal always stand out. Among our isolates, 12 come directly from the collection of samples of this type of infections: 7 wound exudates and 5 abscesses. It is not uncommon for these infections to be complicated by bacteremia due to hematogenous dissemination or arthritis or tenosynovitis due to contiguous dissemination. It is much less common for distant dissemination complication to result in meningitis or arthritis far from the point of inoculation.

Whenever a wound infection occurs that was caused directly by a domestic animal or through objects in contact with them, the involvement of zoonotic microorganisms such as *Bartonella* spp. or *Pasteurella* spp. should be suspected, where the former has a higher incidence but nevertheless its growth on culture media used in clinical practice is more difficult than for *Pasteurella* spp., so that, although we can never say whether there is an underdiagnosis of skin and soft tissue infections by this microorganism, it seems that the incidence of infection is really low.

There are cases in the literature of *Pasteurella* bacteremia treated adequately with a 14-day course of ceftazidime and single-dose gentamicin, with no recurrence, even in patients with significant underlying pathology (4). In the case of bacteremia, intravenous antibiotic treatment and catheter sealing are highly recommended (10). Bacteremia, as is already known, involve a high morbidity and mortality rate, however, in the literature consulted, no estimate has been found of the percentage of cases of bacteremia or sepsis due to *Pasteurella* spp. that end in exitus. It would be interesting to carry out a study on this, as it is an emerging micro-organism. However, due to the low volume of samples we have in our area, due to a population of less than 150,000 inhabitants, it does not seem possible to carry it out. It would be of great interest if the epidemiological study of all these low-incidence micro-organisms were carried out by means of multi-centre studies.

If we consider the wide range of possible infections of different locations that can occur, we can find that they occur more frequently in patients with comorbidities such as diabetes, alcoholism, cirrhosis, cancer, asplenia or prolonged use of corticosteroids (3). However, the underlying immunosuppression in this type of comorbidity is not exclusive to *Pasteurella* spp., but rather implies a greater susceptibility to bacterial infection in different locations and with different pathogens. This is why it is more frequent to find it in this type of patient, but it will always be more frequent to find any type of bacterial infection in them, regardless of virulence factors or pathogenicity.

We found isolation of *P. multocida* in 2 joint fluids, one in 2013 and the other in 2015. Zoonotic intra-articular infections by various microorganisms, such as *Brucella* spp. *Salmonella* spp.

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Campylobacter spp. and *Streptococcus suis*, have been reported, especially in prosthetic joint infections (11)(12)(13)(14). Tenosynovitis and osteomyelitis caused by *Pasteurella* spp. usually originate in an episode of direct traumatic inoculation through the teeth and nails of the animal, and from there, like any other type of infection, it can pass into the bloodstream and cause disseminated infections such as septicemia, meningitis, or pneumonia (15). In our cases it is not known whether the joints had prostheses, whether there was a nearby bite or scratch or whether there was distant dissemination, which would have been interesting to address, and would have provided a more specific and adequate view of the local epidemiology to be considered in joint infections in our area. However, prosthetic infection by *P. multocida* is still an uncommon phenomenon, with fewer than 30 cases described in the literature, and is associated with the presence of comorbidities, notably diabetes and immunosuppressive treatment, among others, as well as pet ownership (16)(17)(18)(19)(20).

Pasteurella joint infections are generally mono-microbial and respond well to treatment with ampicillin and doxycycline, although most of the time treatment had to be accompanied by appropriate replacement of the prosthesis if present. Unfortunately, the antibiogram panel available in the laboratory did not include doxycycline, so we do not know the sensitivity percentage of our strains, whereas, to ampicillin, 18 (81.2%) were sensitive and only one resistant (4.5%). *P. multocida* is usually sensitive to beta-lactams, tetracyclines and co-trimoxazole, with variable resistance to erythromycin and 50% of the strains are resistant to clarithromycin. Beta-lactamase producing strains have been described (21). Few beta-lactam resistances were detected in our isolates. Sensitivity to erythromycin and clarithromycin was not tested.

Infections of other, let's call them non-traumatic, types, even if there is contact with an animal, such as respiratory tract infections or urinary tract infections, are rare. In these cases, the individual has been colonized by close and continuous contact with the pet, through saliva, either by licking or by playing with and chewing on external devices carried by the patient, such as urinary catheters.

In our series, we only found one isolate from a respiratory sample, a sputum, because *Pasteurella* spp. infection of the upper respiratory tract is exceptional, usually found in carriers or contamination in patients in contact with farm or domestic animals, and it is rare for these patients to have underlying respiratory pathology or immunosuppression (22), unlike other infections already described, where the underlying pathology seems to have a certain determining power. Another pathology rarely caused by *Pasteurella* spp. is endocarditis (22).

The literature does not include a study of the possible clinical significance of the isolation of *Pasteurella* spp. in the faeces of a patient; however, in our area we found *P. pneumotropica* predominantly in a stool culture of a patient with diarrhea of short duration and close contact with animals. It remains to be clarified whether this was really the micro-organism causing this pathology or whether it was only a reflection of colonization by this micro-organism in this patient, since no antibiotherapy was given and the symptoms subsided in a few days without further complications, as is usually the case in most infectious gastroenteritis. It is known that through contact with pets *Pasteurella* spp. can colonise the skin of its owner, even colonising the perineal area, and from there it can enter the urinary tract and cause UTIs in predisposed patients. However, it is not known whether it is possible for human oropharyngeal colonisation to pass from there to the gastrointestinal tract and cause acute bacterial gastroenteritis through some pathogenic mechanism or whether it colonises the gastrointestinal tract and appears as the predominant micro-organism in a dysbacteriosis of another cause, such as when staphylococcal or yeast overgrowth is found. This is a very interesting case of *P. pneumotropica* infection, which if more information had been collected would have been published as a separate case report. No further information on the episode could be obtained as it was a single visit of the patient to the hospital emergency department.

It was not possible to collect reliable information about antibiotic regimens as most of the patients completed antibiotic treatment at home, as these were not invasive infections, and therefore no information about these has been added to the study. However, all isolates appeared to be broadly sensitive to beta-lactams and aminoglycosides, so intravenous treatment would rarely be necessary, except in cases of invasive infections such as bacteraemia. The 3 occasions on which fosfomycin was tested correspond to the 3 isolates with significant counts from urine culture, so it seems necessary

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to continue testing this antibiotic in subsequent isolates to find out the true percentage of sensitivity of *Pasteurella* spp. to fosfomycin, since it seems that with 4 (18.2%) episodes of UTI out of the total number of isolates by this family of microorganisms (3 detected by urine culture and another by blood culture) it does not seem to be a particularly infrequent uropathogen, contrary to what is reported in the existing literature (2)(6)(23).

In this study, we found that despite the wide spectrum of infections caused by *Pasteurella* spp. there are only less than 20 cases of UTI in the literature, most of them in patients with urological pathology, especially anatomical alterations (6) and only by *P. multocida* (24), while in our area we also found other species of *Pasteurella* spp. There is a known case of a UTI due to *P. aerogenes* in an 11-year-old girl with a history of neurogenic bladder and in contact with a rabbit, although this species seems to be more frequently related to contact with pigs (25). However, the identification of the bacterial species was performed by biochemical panel tests, so a more reliable identification result would have required the use of other more sensitive techniques that were not available at that time, such as mass spectrometry or rRNA 16S sequencing. However, it appears that dulcitol and sorbitol fermentations are quite useful to differentiate subspecies of *P. multocida*, where *P. multocida multocida* is dulcitol positive and sorbitol negative, while *P. multocida septica* is negative for the fermentation of both sugars. The panels available in the laboratory did not have the dulcitol fermentation test available. It seems that not only exposure to pets accompanied by anatomical alterations of the urinary tract is predisposing for UTI by this micro-organism, but also the presence of urinary catheters, diabetes, or vascular complications, although further studies are clearly needed. They could also be due to non-traumatic contact with pets in which the perineal area is colonised and from there penetrates the urinary tract through the urethral meatus (24) (26) (23) (6). It would be interesting to investigate the possible reasons why there seems to be a higher incidence of UTI due to *Pasteurella* spp. in ASEF than in other areas, whether due to the rurality of the area, the large number of individuals with pets or for any other reason.

As empirical treatment for bites, and especially if *Pasteurella* spp. is suspected, amoxicillin/clavulanic acid is usually recommended, amoxicillin/clavulanic acid is usually recommended, which in our series would have been effective in all but one isolate, with a sensitivity of 95.5% in our case, but, in addition, for *Pasteurella* spp. it is recommended to use as an alternative other antibiotics with good activity such as doxycycline (not tested in our case as already mentioned), trimethoprim/sulfamethoxazole (86. 4% sensitivity in our isolates), penicillin (extrapolated to the sensitivity obtained for ampicillin), cefuroxime (95.5% sensitivity), ciprofloxacin (95.5% sensitivity) or clindamycin (also not tested in our laboratory as it was not included in the panel). Among our isolates, the highest sensitivity of 100% was found for two antibiotics that are not generally recommended for the treatment of this type of infection, gentamicin and cefepime. In any case, gentamicin could be useful in cases of bacteremia, endocarditis or meningitis in combination therapy with a beta-lactam. Cefepime is an antibiotic recommended as a treatment for AmpC beta-lactamase-producing *Enterobacteriaceae* infections, the use of which in these cases makes no sense. Empirical use of erythromycin is not recommended. However, as in all infections, it is recommended that treatment be reconsidered for appropriateness after culture and antibiogram. In addition, often, when an abscess or other purulent collection has formed, the prognosis of the infection will depend on drainage, debridement or even reconstruction if necessary.

In general, beta-lactams, especially carbapenems and cephalosporins, have very good sensitivity to *Pasteurella* spp., which is beneficial in that many can be given orally so that the patient, if conditions permit, can treat at home or parenteral antibiotic regimens can be used, if required, which are well known to clinicians.

It should be borne in mind that many infections due to violent contact with an animal, especially if they are skin or soft tissue infections, will often be polymicrobial, including anaerobic bacteria, so empirical treatment with combination therapy or amoxicillin-clavulanate will be the treatment of choice, so that *Pasteurella* spp. will generally be covered. In addition, often when an abscess or other purulent collection has formed, good prognosis of the infection will depend on drainage, debridement or even reconstruction if necessary.

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With regard to this brief study, it is regrettable that a more recent description of the infections caused by *Pasteurella* spp. in the health area of Ibiza and Formentera (Pitiusas Islands) could not be carried out, but we will try to carry it out soon, either as a new study period or covering both. Furthermore, due to the change in the laboratory's computer system, many data that could have been interesting to know are not recoverable now. Another problem, due to the small size of the laboratory, since it is really the microbiology laboratory of a regional hospital, is the inability to carry out genotyping studies of the isolates obtained, since the priority for sending to reference centres that can do so is given to multi-resistant microorganisms, carbapenemase producers, or those that produce invasive infections of important epidemiological relevance, such as pneumococci or meningococci. In addition, we do not yet have a research support group that would support us with a budget to perform this type of genotyping on demand or that would allow us to purchase E-test strips or extra panels other than those usually used in clinical practice to test antibiotic sensitivity to other antimicrobials.

4. Materials and Methods

This is a retrospective observational study covering the period from 1 January 2013 to 31 December 2018 during which, through the computer system of the Microbiology laboratory (CSM) of the Hospital Can Misses (Ibiza, Balearic Islands), which covers the entire hospital and primary care area of Ibiza and Formentera (ASEF), known as Pitiusas Islands all data were extracted from patients in whom *Pasteurella* spp. was isolated in at least one sample, as well as the possible focus or origin of the infection through consultation of the clinical history. Bacterial identification was performed by automated biochemical test panel Vitek 2 (bioMérieux, Spain). The antibiograms were performed using E-test strips or antibiotic discs (bioMèrieux, Spain) on Mueller-Hinton blood agar plates, incubated in an aerobic atmosphere, at 35-37ºC for 48h. Antibiotic sensitivity was interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards, 2022 version. Medical records were systematically reviewed by collecting demographic data of infected patients, epidemiological data and possible clinical focus of infection.

5. Conclusions

Despite its low prevalence, infection by *Pasteurella* spp. is of great relevance due to the complications it entails, being necessary to apply invasive procedures such as cleaning or surgical drains in more than half of the cases due to its involvement in skin and soft tissue infection.

The species most commonly isolated in our environment is *P. multocida*, which is consistent with the existing literature on human infections. It was not known which subspecies of *P. multocida* was involved, which would be interesting to know in future studies.

Close contact with companion animals, especially cats and dogs, is the main risk factor for *Pasteurella* spp. infections, although in many cases such contact cannot be established. Underlying pathology such as diabetes, cirrhosis or treatment with immunomodulatory drugs may be a predisposing factor in some of these infections, and underlying urological pathology is a risk factor for UTIs due to *Pasteurella* spp.

Pasteurella spp. infection should be considered a zoonosis in possible emergency, especially considering the large proportion of the population with pets, even more so in a predominantly rural area such as Ibiza and Formentera.

Funding: This research received no external funding.

Institutional Review Board Statement: The study protocol complied with the principles of the Helsinki Declaration and ethical epidemiological research. No additional sampling was conducted by the laboratory, and the routine diagnostic protocol was always followed. In this non-interventionist study, biological material was only used for the standard as ordered by attending physicians, with no change in routine procedures. For this reason, there was no need to obtain the informed consent of patients for global results analysis, in accordance with the ethical guidelines of the World Health Organization for health-related research in humans. The

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database was fully anonymized. Permission to access and analyze the data was granted by the Clinical Microbiology Department Management Unit.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The author declares no conflict of interest.

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