Moreover, although we have not found any references in the literature to clinical isolates of KPC-producing *Aeromonas* spp., strains carrying the *blaKPC-2* gene have been reported in a wastewater treatment plant in Japan, so we should be alert to the emergence of these plasmid resistance mechanisms in *Aeromonas* spp.[8]

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Phenotypic and genotypic characterization of *Shigella sonnei* carrying the extended-spectrum beta-lactamase CTX-M-27. A report of two cases in Spain in men who have sex with men

Caracterización fenotípica y genotípica de Shigella sonnei *portadora de la betalactamasa de espectro extendido CTX-M-27. A propósito de dos casos en España en hombres que tienen sexo con hombres*

Case No.1

This was a 28-year-old patient from Portugal who had been in Spain for 15 days and had sexual relations with men (men who have sex with men [MSM]). He went to the Accident and Emergency department with diarrhoea and a fever of 38.1°C, plus mild dysuria and leucocytosis of 20×10^3 μl in blood. Stool and urine samples were collected, and the patient was admitted to the hospital and given intravenous ceftriaxone (1 g/24 h) as empirical treatment. *Shigella* spp. was isolated in the stool culture, and the patient was discharged with oral ciprofloxacin (500 mg/12 h for 3 days) before the susceptibility to antimicrobials had been determined. The isolate turned out to be a group 9 CTX-M extended-spectrum β-lactamase (ESBL)-producing *Shigella sonnei (S. sonnei)*, resistant to cephalosporins, cotrimoxazole and quinolones. Several days later, the patient was admitted again due to persistent symptoms of high fever and watery diarrhoea, this time being treated with ertapenem (1 g every 24 h intravenously for 5 days). He was discharged at the end of the course of antibiotics due to resolution of the symptoms.

Case No.2

This was a 47-year-old patient, MSM, taking ongoing treatment with pre-exposure prophylaxis (PrEP) who attended the sexually transmitted infection (STI) clinic for follow-up and to receive prophylaxis with ceftriaxone (1 g intramuscularly) due to risky sexual contact. He reported a 48-h history of symptoms of diffuse abdominal pain with increased bowel movements (up to 10 per day) with pathological products (mucus/blood), but at that point a watch and wait approach was adopted. He was seen again seven days later and, as the symptoms persisted, stool culture samples were collected from the patient and his current partner (with similar gastrointestinal symptoms) and active monitoring was continued while awaiting the results. In the stool sample, group CTX-M-9, cephalosporinase-type ESBL-producing *S. sonnei* was detected, while non-ESBL-producing *Shigella flexneri* (*S. flexneri)*, whose sensitivity to antimicrobials was different, was detected in its partner (only resistant to quinolones).

Microbiology

In the first case, a PCR panel STI Essential Assay, Allplex™ (Seegene Inc., Seoul, South Korea) was performed on the urine sample (first-catch sample) with detection of *Chlamydia trachomatis*. In both cases, a stool sample was extracted to perform the Enteric bacterial PCR panel of the BD Max™ system (Becton Dickinson, Franklin Lakes, NJ, USA) with detection of enteroinvasive *Shigella/Escherichia coli (E. coli)* DNA. Subsequently, the stool sample was seeded on MacConkey and Hektoen (BD™) agar media, with lactose-negative colonies isolated on the MacConkey agar which were identified as *Shigella* spp./*E. coli* by MALDI-TOF mass spectrometry (Bruker, Massachusetts, USA); this system is not able to distinguish between these two genera. Lactose-negative colonies were plated on triple sugar iron (TSI) agar and lysine iron agar (LIA) (BD™) media for identification, confirming the genus *Shigella* spp. (Fig. 1A). Agglutination was then performed with Difco™ Shigella Antisera Poly (BD™) to determine the species, and the identification of *S. sonnei* was confirmed.

The antibiogram was performed using the disc-diffusion technique, as well as the ID/NMIC 503 panel of the Phoenix BD® system (Becton Dickinson, Franklin Lakes, NJ, USA), through which resistance was detected to cephalosporins, aztreonam, trimethoprim/sulfamethoxazole and quinolones. Susceptibility to azithromycin was also studied in both isolates using E-test®, showing resistance with MIC > 256 mg/l (epidemiological cut-off point for wild strains at ≤16 mg/l). We performed a

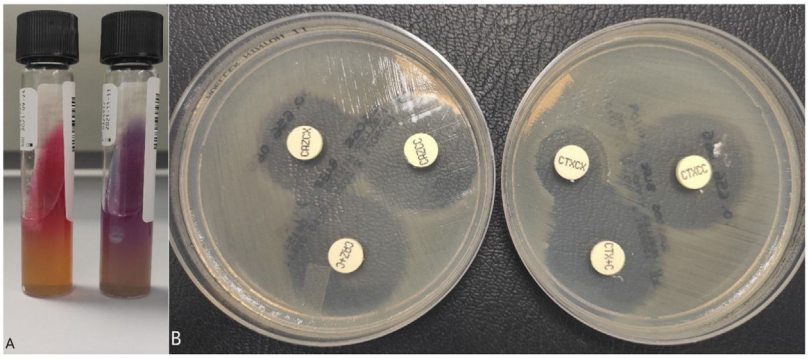

Figure 1. Identification of *Shigella* spp. and detection of the presence of an extended-spectrum beta-lactamase (ESBL).
(A) Left: TSI (triple sugar-iron) medium, glucose fermentation can be seen but not producing gas; right: LIA (lysine-iron agar) medium, there is no decarboxylation or deamination of the lysine. (B) Disc-diffusion technique with the confirmation kits Ïtotal ESBL, AmpC and ESBL + AmpC Confirm kit(ROSCO Diagnostica A/S, Taastrup, Denmark). The increase in the cefotaxime and ceftazidime halo (>5 mm) can be seen in the presence of clavulanic acid but not in cloxacillin, confirming the presence of ESBL.

rapid immunochromatography test (ÑG-Test CTX-M Multiple; NG Biotech, Guipry–Messac, France), which enables detection of the most common types of cephalosporinases (groups CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9 and CTX-M-25), and the result was positive. The ESBL resistance phenotype was then confirmed using the disc-diffusion technique with the confirmation kits (Fig. 1B) Ïtotal ESBL, AmpC and ESBL + AmpC Confirm kit(ROSCO Diagnostica A/S, Taastrup, Denmark) and the Čheck-Direct ESBL Screenpanel of the BD Max™ system with detection of group 9 CTX-M cephalosporinase-type beta-lactamase (which can include different genes: *blaCTX-M-14*, *blaCTX-M-24* and *blaCTX-M-27*).

Both strains were sent to the National Centre for Microbiology (Instituto de Salud Carlos III, Majadahonda, Madrid, Spain) which performed phenotypic and genotypic detection of antibiotic resistance. Both strains were detected as being carriers of CTX-M-27 ESBL by phenotypic techniques and by sequencing, belonging to the 152 sequence (MLST). The analysis of the genomes indicated that both isolates belonged to the cluster from the multi-drug resistant *S. sonnei* alert issued by the United Kingdom in the months prior to the exposed cases.[1] The two isolates were identical to each other within 0 alleles using the cgMLST scheme of the SeqSphere software (Ridom Bioinformatics, Germany).

Discussion

The genus *Shigella* (includes the species *S. sonnei, S. flexneri, Shigella boydii* and *Shigella dysenteriae)* causes a gastrointestinal syndrome called shigellosis. Most patients develop diarrhoea (sometimes with blood and/or mucus), fever and abdominal pain. Transmission occurs mainly by person-to-person contact with contaminated food or water. The infection is normally self-limiting, without the need for antibiotic treatment. As far as sexual transmission is concerned, in recent years it has been associated with outbreaks among MSM throughout the world, in the USA and Spain and in London in the UK.[2–4] Regarding infection risk factors, it is possible that the use of PrEP could be influencing sexual behaviour by reducing the use of barrier measures (condoms), thus increasing the prevalence of STI, including shigellosis as an emerging cause.[5]

Resistance to antimicrobials due to ESBL is becoming a major problem. There have been very few reports to date of ESBL-producing *S. sonnei* strains in patients who have not travelled to endemic areas (Asia), so an adequate surveillance programme could provide us with information about the epidemiological situation here in Spain.[6–8] Specifically in the UK, several outbreaks (or a prolonged outbreak) of ESBL-producing *S. sonnei* ($bla_{\text{CTX-M-27}}$-Group 9 gene) and a QnrB19 plasmid (reduced sensitivity to quinolones) have been reported among MSM since 2015.[9] In Australia an increase has been noted since 2019 in cases of shigellosis among MSM whose strains share a profile similar to those studied in the UK (probably introduced by travellers who returned from an area where this clonal group was circulating).[10] In Spain, there are eight confirmed cases and 22 possible cases related to these multi-drug resistant strains of *S. sonnei*. In fact, at least four other sequenced isolates, and our two strains, are closely related within the cluster and to representative sequences from the UK.[1]

In MSM patients with gastrointestinal symptoms including fever, abdominal pain, vomiting or diarrhoea with pathological products such as blood or mucus, *Shigella* spp. should be suspected as a sexually transmitted infection. The use of antibiotics should be restricted to moderate/severe cases to avoid the consolidation and transmission of antibiotic resistance. From an epidemiological point of view, transmission should be monitored and prevention measures should be taken as with any other STI, avoiding sexual intercourse and doing a repeat stool culture to ensure that the bacterial load is low or undetectable.

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Synergistic anti-malarial effects of *Ocimum sanctum* leaf extract and artemisinin

Efectos antipalúdicos sinérgicos del extracto de hoja de Ocimum sanctum *y artemisinina*

Malaria remains a major health problem in Indonesia. It has been reported that the prevalence of parasitemia in Timika, Papua was 16.3%, and almost 50% of cases were caused by *Plasmodium falciparum* (*P. falciparum*).[1] Papua province has not only the highest prevalence of malaria in Indonesia but also the highest prevalence of multidrug-resistance to both *P. vivax* and *P. falciparum*.[2] Although artemisinin-based combination therapy (ACT) was adopted as the first-line anti-malarial treatment, studies have demonstrated the failure of ACT towards malaria elimination in several Southeast Asian countries.[3] Hence, an alternative combination therapy against malaria is needed.

Ocimum sanctum (*O. sanctum*, Indonesian name Kemangi) belongs to the Lamiaceae family and is widely distributed throughout Indonesia. *O. sanctum* is used by Indonesian to treat several diseases, including malaria; however, the underlying mechanism remains elusive. A study revealed that the ethanolic leaf extract of *O. sanctum* displays more potent antiplasmodial activity than other Ocimum species *in vitro*.[4] Therefore, this study aims to evaluate the synergistic anti-malarial properties of *O. sanctum* and artemisinin (ART) against the *Plasmodium* infection *in vivo*. In addition, the level of transforming growth factor-beta (TGF-β) was examined.

The leaves of *O. sanctum* were collected from Malang, East Java, Indonesia. The species was identified and confirmed by a plant taxonomist of the herbarium unit, UPT Materia Medica, Batu, East Java, Indonesia. The ethanolic extract preparation was conducted as previously described.[5] For *in vivo* experiments, female Balb/c mice between 8 and 12 weeks of age were used. Sixty-three mice were randomly assigned into seven groups (9 mice per group), namely negative control, positive control, infected mice treated with ART (0.036 mg/g/day); two different doses of *O. Sanctum* extract (0.25 and 0.5 mg/g/day); and combinations of artemisinin and *O. Sanctum* extract. The malaria model was performed by i.p. injection of *Plasmodium berghei* adjusted to 10^6 parasites in 0.2 mL blood per mouse. Infected mice were then treated on the sixth day of infection with parasitemia approximately around 10% for seven consecutive days. To examine TGF-β levels, mice peritoneal macrophages on day seven post-treatment were cultured as described previously,[6] and the supernatants were used to quantify the concentration of TGF-β (BioLegend) by ELISA.[7–10] The study was approved by the medical ethics committee of Brawijaya University with Reference No. 27-KE. The reduction of parasitemia and TGF-β levels were analyzed by two and one-way ANOVA, respectively, followed by the Fisher LSD *post hoc* test using StatPlus. Significant differences were accepted when $p < 0.05$.

The reduction of parasitemia was observed in all groups started on day five of the treatment. Notably, the administration of ART and *O. Sanctum* extract at a dose of 0.5 mg/g/day demonstrated a higher effectivity to speed up *Plasmodium* clearance than other groups (at day 3 compared to the baseline level, Fig. 1A). Moreover, the suppression of TGF-β was only observed in mice treated with combination therapy but not with monotherapy (Fig. 1B), thereby implying that combination therapy exhibits synergistic anti-malarial effects towards *Plasmodium* elimination. Various active constituents have been identified in the ethanolic extract of *O. Sanctum*, such as alkaloids, glycosides, flavonoids, phenols, saponins, tannins, steroids, and triterpenoids.[4] The possible mechanisms of *O. Sanctum* extract in eliminating *P. berghei* may have occurred through the inhibition of hemozoin biocrystallization, protein synthesis, or β-haematin formation, stimulation of DNA fragmentation, and cytoplasmic acidification.[4]

In line with previous findings, this study showed that the upregulation of TGF-β levels was observed in *P. berghei* infected mice. Furthermore, the upregulation of TGF-β is known to be associated with the risk of complicated malaria.[11] These results imply that TGF-β is linked to the disease severity of malaria. Therefore, a treatment that modulates the suppression of TGF-β would be beneficial to minimize malaria progression. In summary, the combination of ART and the ethanolic extract of *O. Sanctum* displays synergistic anti-plasmodial activity *in vivo*. Further studies are warranted to investigate the potential of *O. Sanctum* as an alternative ACT regimen in clinical settings.

Authors' contribution

Z.S.U. conceived, performed, analyzed, and wrote the article.

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