Doña, I.; Pérez-Sánchez, N.; Salas, M.; Barrionuevo, E.; Ruiz-San Francisco, A.; Hernández Fernández De Rojas, D.; Martí-Garrido, J.... (2020). Clinical Characterization and Diagnostic Approaches for Patients Reporting Hypersensitivity
Reactions to Quinolones. Journal of Allergy and Clinical Immunology: In Practice. 8(8):2707-2714. https://doi.org/10.1016/j.jaip.2020.04.051

TITLE: Clinical characterization and diagnostic approaches for patients reporting hypersensitivity reactions to quinolones

SHORT TITLE: Characterization and diagnosis of hypersensitivity reactions to quinolones.

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Total word count for the text: 35864 words.
Total word count for the Abstract: 25048 words.

Conflict of interest: None of the authors has any conflict of interest or received any money for the present study. Research is part of their daily activities. All the authors had full access to all the data and can take responsibility for the integrity of the data and the accuracy of the data analysis.

Funding: The present study has been supported by the Institute of Health "Carlos III" of the Ministry of Economy and Competitiveness (grants cofounded by European Regional Development Fund (ERDF): RETIC ARADYALRD16/0006/0001, RD16/0006/0010, RD16/0006/0019 and RD16/0006/0030. I Doña is a Clinical Investigator (B-0001-2017) from Consejería de Salud of the Andalusian government, Junta de Andalucía. N Pérez-Sánchez holds a Rio Hortega research contract (CM17/0014), and E Barrionuevo a Juan Rodes research contract (JR18/00049), both from the Institute of Health "Carlos III", Spanish Ministry of Economy and Competitiveness (grants cofounded by European Social Fund, ESF).

ABSTRACT

Background: Quinolones are the second most frequent cause of hypersensitivity reactions (HSRs) to antibiotics ~~after betalactams~~. A marked increase in the number of patients with HSRs to quinolones has been detected.

Objective: To describe the clinical characteristics of patients with HSRs to quinolones and present methods for their diagnosis.

Methods: Patients attending the allergy unit due to reactions suggestive of HSRs to quinolones were prospectively evaluated between 2005-2018. Diagnosis was achieved using clinical history, skin tests (STs), ~~the~~ basophil activation tests (BATs), and drug provocation tests (DPTs) if ST and BAT were negative~~as necessary~~.

Results: We included 128 subjects confirmed as having HSRs to quinolones and 42 found to be tolerant. Anaphylaxis was the most frequent entity in immediate HSRs and was most commonly induced by moxifloxacin. Patients were evaluated a median of 150 days (interquartile range: 60-365) after the reaction. Of patients who underwent ST and BAT, 40.7~~4~~% and 70% respectively were positive. DPT with a quinolone was performed in 48 cases, giving ~~different~~ results depending on the culprit drug: when moxifloxacin was involved~~the culprit~~, 62.5% of patients gave a positive DPT to ciprofloxacin, whilst none reacted to levofloxacin. The risk of HSR was 96 times higher in subjects who reported moxifloxacin-induced anaphylaxis and 18 times higher in those reporting immediate reactions compared to clinical entities induced by quinolones other than moxifloxacin and non-immediate reactions.

Conclusions: The diagnosis of HSR to quinolones is complex. The use of clinical history is essential as a first step. BAT shows higher sensitivity than STs. DPTs can be useful for finding safe alternative quinolones.

What is already known about this topic?

Quinolones can induce hypersensitivity through several mechanisms, being the third most common drug associated with hypersensitivity, and the second most frequent drug inducing both IgE-mediated hypersensitivity and severe anaphylaxis. The optimal diagnostic approach remains a controversial topic.

What does this article add to our knowledge?

The risk of having quinolone hypersensitivity is higher for immediate reactions, particularly for moxifloxacin-induced anaphylaxis. The basophil activation test has a higher sensitivity than skin test. Drug provocation testing can be useful to identify safe alternative quinolones.

How does this study impact current management guidelines

We propose an algorithm for diagnosing quinolone-induced reactions, which should be classified according to the interval between drug intake and reaction onset, using a 6 hour threshold. The algorithm includes skin, basophil activation, and drug provocation tests as necessary.

KEYWORDS: Adverse drug reaction; Anaphylaxis; Basophil activation test; Ciprofloxacin; Drug provocation test; Hypersensitivity; Levofloxacin; Moxifloxacin; Quinolones; Skin tests.

ABBREVIATIONS: Acute generalized exanthematous pustulosis (AGEP); Basophil activation test (BAT); Drug provocation test (DPT); Fixed drug eruption (FDE); Hypersensitivity reaction (HSR); Immediate reaction (IR); Intravenous (IV); Maculopapular exanthema (MPE); Non-immediate reaction (NIR); Skin prick test (SPT).

INTRODUCTION

Quinolones are antibiotics that are commonly prescribed for their effectiveness against Gram (+) and Gram (-) bacteria[1-3]. Adverse effects occur in 2-10% of people taking quinolones, however most of them are mild, mainly affecting the gastrointestinal or central nervous systems[3,4]. Quinolones can induce hypersensitivity reactions (HSRs)[5] through IgE-mediated reactions (immediate reactions, IRs) and T-cell dependent reactions (non-immediate reactions, NIRs)[6,7]. In addition, quinolones may also cause HSRs in drug-naïve patients[8,9]. A mechanism of mast cell activation via occupation of the human Mast-related G-protein receptor X2 (MRGPRX2) has been described for IRs[10,11] occurring in patients without previous exposure to quinolones[12].

Although the absolute risk of an HSR related to quinolones is low (44.0 (95% CI: 34.8–53.3) emergency department visits/100,000 prescriptions)[8], quinolones are the third most frequent drug associated with HSRs[13] in general, and the second most frequent in IgE-mediated HSRs. They are also the second most frequent cause of alert activation for antibiotic allergy in electronic hospital records[14] and severe drug-induced anaphylaxis[13,15]. In recent years, an increase in the percentage of patients with HSRs to these drugs has been detected, ranging from 0.54% in 2005 to 6.85% in 2010[13]. This is likely due to their increased prescription over the last decades[16]. The incidence of anaphylaxis induced by quinolones has been estimated to be 1.8–23 per 10 million days of treatment[17,18] and the prevalence of cutaneous adverse reaction to be 0.09%[19]. Quinolones are also one of the main triggers of acute generalized exanthematous pustulosis (AGEP), photosensitivity and vasculitis[20].

HSRs to quinolones appear in an important percentage of patients (23%) previously diagnosed as allergic to betalactams: in fact, betalactam allergic patients have a 17 times higher risk of reacting to quinolones than those non-allergic. This represents an important health problem as it greatly decreases therapeutic options availables[16]. In a large study of inpatients with common infections requiring antibiotic treatment, quinolone allergy occurred in 5.4% of patients who were already sensitive to betalactams[21], leading to important restrictions for antibiotic prescription and subsequently poor prognosis of their infections.

The optimal diagnosis of quinolone HSRs is still a matter of debate. The value of skin tests (STs) is uncertain, and they have shown false positive results when quinolones are tested at high concentration[22-25]. The presence of specific IgE to quinolones has been reported using the sepharose radioimmunoassay, with a sensitivity of 54.5%[26]. The

basophil activation test (BAT) has shown promising results for the diagnosis of patients with IRs to quinolones[27-29]. However, other studies have contradicted these findings[30,31]. The gold standard, therefore, is the drug provocation test (DPT). However, this is not free of risk and not advisable in cases where the reaction might be severe.

The aim of this study was to describe the clinical characteristics of a large group of patients with quinolone-induced HSRs and present methods for their diagnosis.

METHODS

Patients

We prospectively evaluated patients with symptoms suggestive of HSR to quinolones that had been referred to the Allergy unit of the University Regional Hospital of Málaga, University Hospital of Salamanca, and of the University Hospital La Fe of Valencia over a period of 13 years (2005-2018).

Inclusion criteria. Patients ≥14 years-old in whom the allergological study was completed were included and classified in two groups: A) Patients confirmed as having HSRs to quinolones (by positive STs, BATs or DPTs); and B) Patients confirmed as being non-allergic (tolerant) as they tolerated a DPT with the suspected culprit quinolone.

Exclusion criteria. Patients <14 years-old; patients in whom the allergological study was not completed so that the diagnosis could not be confirmed as being neither allergic nor tolerant to quinolones: pregnant or breastfeeding patients; patients taking beta-blockers or ACE inhibitors or with contraindications for epinephrine administration; patients who had acute infections and/or underlying cardiac, hepatic or renal diseases that contraindicated DPTs; and subjects with psychosomatic disorders.

Clinical history

Patients were asked about their reaction symptoms[32], the interval between drug intake and reaction onset, the number of episodes, the interval between their last reaction and the study, and the presence of other underlying diseases. If a reported reaction occurred within 6 hours after quinolone intake, the reaction was classified as IR; when this interval was longer, it was considered an NIR[5,32].

Skin testing

For reactions suggestive of an IR, skin prick tests (SPTs) were carried out as described[33] using ciprofloxacin (at 0.02 and 0.2 mg/ml), levofloxacin (at 0.05 and 0.5 mg/ml), and moxifloxacin (one tablet of 400 mg suspended in NaCI). Intradermal tests were not performed to avoid false positive results as non-specific histamine release by quinolones has been reported[34,35].

For reactions suggestive of a~~n~~ NIR, patch tests (PTs) were carried out and evaluated as described[33] by mixing powdered quinolone (ciprofloxacin, levofloxacin, and moxifloxacin) in petrolatum at 30% w/w.

Basophil activation test

In patients with a suspected IR, BATs were performed as described previously[27], using ciprofloxacin (2 and 0.2 mg/ml), levofloxacin (4 and 2 mg/ml), moxifloxacin (2, 0.2 and 0.1 mg/ml), norfloxacin (2, 0.2 and 0.1 mg/ml), ofloxacin (4, 2, 0.2 and 0.1 mg/ml), and lomefloxacin (4, 2, 0.2 and 0.1 mg/ml).

Drug provocation test

DPTs with ciprofloxacin, levofloxacin, and moxifloxacin were performed in a single blind manner if skin tests and BATs were negative[36]: placebo capsules were given at different times on the first day; increasing doses of quinolones were administered orally at intervals of 60 min (5, 20, 100 mg for ciprofloxacin and levofloxacin; 5, 30, 65 mg for moxifloxacin) on the second day. If these did not produce a reaction, three further doses of quinolones were given on the third day: 125, 125, 250 mg (accumulative dose 500 mg) for ciprofloxacin and levofloxacin; 100, 100, 200 mg (accumulative dose 400 mg) for moxifloxacin. The three test days were separated by 1 week. If cutaneous and/or respiratory symptoms or alterations in vital signs appeared, the procedure was stopped and the symptoms were evaluated and treated. If no symptoms appeared during graded challengedrug administration, the therapeutic dose of quinolone was achieved. T and this was then followed by taking the full 2 days at maximum dose at home, starting after a gap of 24 hours after the graded challenge. Before beginning the DPT procedure, patients were stable and their forced expiratory volume in 1s had to be at least 80% of the predicted value, with an absolute volume of at least 1.5 L. Medications were stopped before DPT according to international guidelines[36].

Statistical analysis

Data analysis was performed using Chi-square analysis to test differences in nominal variables between groups, the Fisher test was used when there were no criteria for using the Chi-square test and the Mann–Whitney test was used for quantitative variables. All reported p values represented two-tailed tests, with values <0.05 considered statistically significant. A logistic regression analysis was performed to establish the characteristics associated with the diagnosis of HSR or tolerance to quinolones and with the diagnosis of immediate anaphylaxis. The following variables were analysed: gender, age, time interval between drug intake and the onset of the reaction, symptoms experienced, drugs involved, time interval between drug reaction and study, and number of episodes.

The study was conducted according to the principles of the Declaration of Helsinki. All the participants were informed orally about the study and signed the corresponding informed consent.

RESULTS

A total of 612 patients with a clinical history suggestive of an HSR to quinolones were evaluated. Of these, full diagnosis could not be achieved for 442 patients: 361 patients that gave a negative ST and negative BAT could not undergo DPT to quinolones due to age, comorbidities or because it was contraindicated due to the potential severity of the reaction; 78 did not give consent for the allergological tests (STs, BAT and/or DPTs); and 3 were excluded due to pregnancy. For the remaining 170 patients a full diagnosis could be achieved: 128 were confirmed as having HSRs to quinolones and 42 as non-allergic (tolerant) to quinolones.

Clinical data of the subjects included in the study

The 170 included subjects with confirmed diagnosis had a median age of 53 [interquartile range: 40–63.25] years, and 126 (74.1%) were female. The majority of cases reported only one previous episode induced by quinolone intake, except for 2 cases who reported 2 previous IRs. As such, the patients included in the study reported a total of 172 previous reactions: 120 IRs and 52 NIRs, with the percentage of IRs higher in those confirmed as having HSRs compared to the tolerant group (73.8% vs 19%; $p<0.0001$) (Table 1). Most reported reactions were induced by oral quinolones (142; 82.5%), the rest by intravenous route (30;17.4%). In terms of the symptoms of reported reactions, the percentage of anaphylaxis reactions was higher in subjects confirmed as having HSR ($p<0.0001$); whereas urticaria ($p=0.0004$), local reaction at the site of IV administration ($p=0.0001$) and MPE ($p=0.03$) were more frequently report by subjects that were found to be as tolerant (Table 1). Moxifloxacin was the most frequent culprit quinolone in patients with confirmed HSRs; ciprofloxacin was more frequent in subjects confirmed as tolerant ($p<0.0001$ and $p=0.001$, respectively) (Table 1). In subjects confirmed as having HSRs, most cases of anaphylaxis were induced by moxifloxacin (52.9%; $p=0.002$); urticaria and angioedema were mostly induced by ciprofloxacin (48.8% and 66.7%, respectively), although these differences was not found to be statistically significant (Table E1). For those found to be tolerant, ciprofloxacin was the most frequent cause of both urticaria and angioedema (69.2% and 66.7%, respectively), as well as of local reactions at the site of IV administration (66.7%) ($p>0.05$) (Table E1).

The logistic regression analysis showed that the risk of being confirmed as HSR was higher for cases who reported moxifloxacin-induced anaphylaxis (OR: 96.16; CI: 6.172-Inf; $p=0.009$) and for those reporting IRs (OR: 18.856; CI: 5.196-271.449; $p<0.0001$)

compared to cases who reported other symptoms induced by other quinolones and NIRs. Moreover, the risk for being confirmed as HSR decreased when ciprofloxacin was the culprit (OR: 0.107; CI: 0.002-0.741; p=0.04) and the symptoms reported were MPE, FDE, urticaria, angioedema (OR: 0.053; CI: 0-0.452; p=0.03), or a local reaction at the site of IV administration (OR: 0.001; CI: 0-0.016; p=0.0006). No significant associations were found for the other variables, and there were no interactions between variables.

Analysis of the patients confirmed as suffering HSRs to quinolones

In patients confirmed as having HSRs, a total of 112 reported reactions (73.8%) were IRs and 18 (26.2%) NIRs. No differences were found when comparing age, sex, atopy, allergen sensitization and underlying diseases between IR and NIR groups (data not shown).

Anaphylaxis was the most frequent reported symptom among IRs ($p<0.0001$) and urticaria among NIRs ($p>0.05$) (Table 2). It is of note that the 7 (25%) of patients reporting reactions within the interval of 1-6 hours showed symptoms compatible with anaphylaxis. Moxifloxacin was the most frequent quinolone involved in IRs and ciprofloxacin in NIRs (41.1% and 38.9%, respectively) (Table 2). Anaphylaxis was induced primarily by moxifloxacin in IRs (52.9%; OR=2.935 (IC:1.418-6.075), p=0.003) whereas most urticaria and angioedema was induced by ciprofloxacin (52.9% and 75%, respectively). Considering NIRs, moxifloxacin was the culprit in most cases reporting urticaria (42.8%) and ciprofloxacino in angioedema reporting-cases (50%) (Table E2). The time interval between intake and onset of the reaction was shorter when the drug was administered by an IV route compared to the oral route (5 [IR: 5-10] minutes vs 30 [IR: 15-60] minutes, p=0.005). This comparison was also statistically significant when ciprofloxacin was the culprit (IV route: 5 [5-10] minutes; oral route: 30 [18.7-165] minutes; p=0.01) (Table E3).

Methods used for diagnosis

The median time interval between the reaction and the study was 150 days [interquartile range: 60-365] (mean: 560.3 days, SD: 1028.4 days). No differences were found between IRs and NIRs. STs were performed on 54 subjects, BATs on 76, and DPTs on 48. No differences were found when comparing the clinical characteristics of patients undergoing the different tests (data not shown).

Skin tests

SPTs were performed on 48 patients and PTs on 6, of which 22 were positive: 20 SPTs and 2 PTs. Of the positive SPTs, 13 (43.33%) were positive to moxifloxacin, 7 (8.53%) to ciprofloxacin, and 6 (9.83%) to levofloxacin (Table 3). When ciprofloxacin was the suspected culprit drug, SPTs to ciprofloxacin were positive in 16.7% of the tests and levofloxacin in 22.2%; when levofloxacin was the suspected culprit, 25% of SPTs were positive to levofloxacin and 80% to moxifloxacin. Finally, when the suspected culprit was moxifloxacin, 100% of SPTs were positive to moxifloxacin and 6.2% to ciprofloxacin (Table 3). Regarding the symptomatology of the reported reactions, the highest percentage of positive ST results was found for anaphylaxis (53.8%), followed by urticaria (33.3%). Although the interval between the last quinolone-induced reaction and the study was shorter in patients with positive STs compared to negative, no statistical difference was found (90 [interquartile range: 60-240] vs 120 [interquartile range: 60-172.5] days; p=1).

Basophil activation test

The BAT was positive in 68 (89.5%) of cases. A total of 56 (76.7%) cases were positive to ciprofloxacin, 35 (53.8%) to moxifloxacin, 26 (44.1%) to levofloxacin, 15 (83.3%) to norfloxacin, 10 (58.8%) to ofloxacin and 10 (55.5%) to lomefloxacin (Table 46). When ciprofloxacin was the culprit, BAT to ciprofloxacin was positive in 80%, to moxifloxacin in 60% and to levofloxacin in 47.4%; when levofloxacin was the culprit, BAT was positive to ciprofloxacin in 72.7%, to levofloxacin in 45.4% and to moxifloxacin in 20%; finally, when the culprit drug was moxifloxacin, BAT was positive to ciprofloxacin in 76.5%, to moxifloxacin in 62.5% and to levofloxacin in 46.2% (Table 46). BAT was positive in 48 out of 49 (97.9%) cases reporting anaphylaxis, in 13 out of 18 (72.2%) cases of urticaria and in 5 (100%) cases of angioedema. Although the interval between the historical quinolone reaction and whether the patients were found to be allergic or not in the study was shorter in patients who gave a positive BAT compared to negative, no statistical differences were found (150 [interquartile range: 60-365] vs 395 [interquartile range: 60-1003.7] days; p=0.9909).

Drug provocation test

We performed 58 DPTs in 48 patients. A total of 34 DPTs with the culprit quinolone were carried out in cases with negative ST and BAT, all of which were positive: 23 in IR (16 with ciprofloxacin, 3 with levofloxacin, and 4 with moxifloxacin) and 11 in NIR (5 with ciprofloxacin, 2 with levofloxacin, and 4 with moxifloxacin). When the benefit

was considered to outweigh the risk, we carried out DPTs with an alternative quinolone in 24 cases, this was positive for 13 of these (11 IR and 2 NIR) (Table 5). When ciprofloxacin was the culprit, DPT to levofloxacin was positive in 60%; when levofloxacin was the culprit, DPT was positive to ciprofloxacin in 40%; when the culprit drug was moxifloxacin, DPT was positive to ciprofloxacin in 62.5%, and no case reacted to levofloxacin (Table 5). In all cases DPTs with quinolones induced mild symptoms (pruritus and wheals localized on different parts of the body) that disappeared 1-48 hours after administering antihistamine and corticosteroid treatment.

DISCUSSION

Although hypersensitivity reactions (HSRs) to quinolones represent an important health problem–[21], no large-scale study of patients suffering from them exists. To our knowledge, this is the largest published series of HSRs to quinolones to date. In our study, more than the 50% of patients reported anaphylaxis, most of whom suffered from immediate reactions (IRs), in agreement with previously published short series[24,26,27,30,34]. Moreover, data suggests that the risk of an HSR is different depending on the quinolone. Analyses of spontaneous reports implicate moxifloxacin triggers anaphylaxis in a higher proportion of cases than levofloxacin or ciprofloxacin[8,9,37], which is in line with our results. Indeed, the risk of experiencing anaphylaxis was 2.2-fold higher when moxifloxacin was the culprit, which agrees with previously published data[8,9,37]. This may be due to the expanded use of quinolones or increased immunogenicity to newer quinolones.

The interval between drug intake and the appearance of symptoms is crucial for evaluating allergic reactions to drugs. Historically, reactions occurring less than one hour after drug intake are considered IRs, and those occurring after an hour are considered NIRs[5,38]. The former are thought to be induced by an IgE-mediated response, although an alternative non-IgE dependent mechanism may also be involved–[10,11]. For the latter, the underlying mechanism remains a matter of debate, especially for those with a time interval of 1 to 6 hours after drug intake[39-41]. For betalactam antibiotics, the mechanism has been proposed to be non-IgE dependent, as some evidence suggests that these reactions are T-cell mediated[40-41]. On the other hand, for metamizole, a study of reactions occurring 1–8 hours after intake using basophil activation testing support an IgE-mediated[42]. However, to our knowledge, this mechanism has not yet been studied for quinolones. In this study, we have observed that around 25% of patients reported anaphylaxis 1 hour after quinolone intake, and more than 40% of them showed positive

results via BAT or SPT, suggesting that an IgE mechanism is likely. The interval between drug administration and reaction onset may be related to the production of as-yet unidentified metabolites and the route of administration. However, most patients in our study took the quinolone orally and no differences could be found in terms of administration route when considering drugs involved and symptoms reported. As such, we would suggest that the classification of reactions as IR or NIR based solely on a 1 hour cut-off does not sufficiently reflect the extension of the pathophysiology of the reactions.

The diagnosis of HSRs to quinolones is still a matter of debate. A detailed clinical history is crucial as a first approach. We found that the chance of being confirmed as having an HSR to quinolones was 96 times higher in patients who reported moxifloxacin-induced anaphylaxis and 18 times higher in those reporting IRs. This risk decreased when ciprofloxacin was the culprit and the symptoms experienced in the reported reaction were MPE, FDE, urticaria, angioedema or a local reaction at the site of the administration of the drug. Concerning STs, there is controversy regarding their utility. Some authors consider they are useful, with a sensitivity of 71%, specificity of 86%, and positive and negative predictive values of 50% and 94%, respectively having been reported previously[24]. However, other studies suggest that STs are not valid because they can produce false-negatives[34,35], potentially missing important reactions and putting patients at risk, moreover they can also produce a large number of false-positive results when tested at high concentrations, which is attributed to non-specific histamine release by quinolones due to mast cell activation[35,43-48]. We decided not to perform intradermal tests in our patients based on this consideration. In our study, we found a low sensitivity for STs in general, although it was higher for severe reactions (anaphylaxis) and when levofloxacin and ciprofloxacin were the culprits.

We found the BAT to be useful for the diagnosis of patients with IRs to quinolones[27-29]. However, other studies have shown contradictory results[30,31]. Here, BAT gave a higher percentage of positive results than STs, agreeing with previous studies[27,28]. This is important, because if BATs can be used to confirm diagnosis instead of DPTs in some cases, this will reduce patient risk. This is particularly useful here, given that the most common clinical entity induced by quinolones is anaphylaxis.

Although cross-reactivity among quinolones remains a controversial issue, DPTs could be useful to find safe alternative quinolones. A high degree of cross-reactivity has been reported between the first- and second- generation quinolones[22]. Regarding the second

generation, cross-reactivity does not always occur within this group[43,45,49], –which may be due to the production of different metabolites. The same phenomenon can occur for the newer (moxifloxacin) and the second- (ciprofloxacin) and third-generation (levofloxacin) quinolones[50-52]. A low degree of cross-reactivity has been found between levofloxacin and ciprofloxacin[34]. In our study, 60% of the patients who reported reactions induced by levofloxacin tolerated ciprofloxacin in DPT and 40% of cases tolerated levofloxacin when the reactions were induced by ciprofloxacin. DPT with levofloxacin was carried out for 2 cases who reported moxifloxacin-induced reactions, with neither patient experiencing an adverse reaction. Based on the data obtained from our large series of cases, we propose an algorithm for the diagnosis of quinolone-induced HSRs, as described in Figure 1.

A limitation of this study is the high percentage of patients for whom we were not able to confirm the diagnosis due contraindication or patient refusal. This could be the reason why the number of cases confirmed as tolerant in our series is low. However, despite this, our results show relevant differences in clinical characteristics comparing tolerant and cases confirmed as HSRs, highlighting the importance of a detailed clinical history as an initial approach for diagnosis. Another limitation of our study is that ST, BAT, and DPT could not be performed for all patients and with all quinolones, that PT was carried out at a 30% dilution in petrolatum which could increase the rate of false negative results, and that the time interval between the reaction and the allergy evaluation was not uniform in all patients. However, our aim was to describe the role and utility of the different diagnostic methods performed in a large group of patients in real allergological practice, not finding differences in the clinical characteristics when comparing groups of patients based on results for ST, BAT, and DPT.

The accurate diagnosis of quinolone-induced HSRs is an important issue not only due to their frequency, as described above, but also due to the fact that an important percentage of patients that report quinolone-induced HSRs report previous reactions to betalactams, drastically reducing their therapeutic options[16]. Referring patients with quinolone-induced HSRs for a full allergological evaluation is crucial to confirm or dismiss their reported allergy.

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Table 1. Clinical data for the reactions reported by the subjects included in the study, comparing cases confirmed as having HSRs to quinolones and those confirmed as tolerant to these drugs. AE: Angioedema. FDE: Fixed drug eruption. HSR: Hypersensitivity reaction. MPE: Maculopapular exanthema.

		HSR n=130	Tolerant n=42	p
Historical reaction symptoms; n (%)	Anaphylaxis	70 (53.8)	-	<0.0001
	Urticaria	41 (31.5)	26 (61.9)	0.0004
	AE	12 (9.2)	6 (14.3)	0.3522
	FDE	4 (3.1)	-	0.5732
	MPE	3 (2.3)	4 (9.5)	0.03
	Local reaction at the site of intravenous administration	-	6 (14.3)	0.0001
Drugs involved in historical reactions; n (%)	Ciprofloxacin	49 (37.7)	28 (66.7)	0.001
	Levofloxacin	21 (16.2)	10 (23.8)	0.2618
	Moxifloxacin	52 (40)	2 (4.8)	<0.0001
	Norfloxacin	3 (2.3)	2 (4.8)	0.5967
	Ofloxacin	1 (0.8)	-	1
	Pipemidic acid	2 (1.5)	-	1
	Unknown	2 (1.5)	-	1

Time interval intake-reaction, median (IR) (min)		30 (11.25-60)	7200 (2880-8640)	<0.0001
Time interval intake-reaction ≤1h; n (%) Time interval intake-reaction >1h; n (%)		112 (73.8) 18 (26.2)	8 (19) 34 (81)	<0.0001
Administration route; n (%)	Oral Intravenous	109 (83.8) 21 (16.1)	33 (78.6) 9 (21.4)	0.4335
Number of episodes, median (IR)		1 (1-1)	1 (1-1)	0.08

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Table 2. Clinical data for the reported reactions in cases confirmed as HSRs to quinolones. AE: Angioedema. FDE: Fixed drug eruption. MPE: Maculopapular exanthema.

		Immediate n (%)	Non-immediate n (%)	p
Historical reaction symptoms; n (%)	Anaphylaxis	70 (62.5)	-	<0.0001
	Urticaria	34 (30.4)	7 (38.9)	0.4696
	AE	8 (7.1)	4 (22.2)	0.04
	FDE	-	4 (22.2)	0.0002
	MPE	-	3 (16.7)	0.002
Drugs involved in historical reaction; n (%)	Ciprofloxacin	42 (37.5)	7 (38.9)	0.9101
	Levofloxacin	17 (15.2)	4 (22.2)	0.451
	Moxifloxacin	46 (41.1)	6 (33.3)	0.5339
	Norfloxacin	2 (1.8)	1 (5.6)	0.3629
	Ofloxacin	1 (0.9)	-	1
	Pipemidic acid	2 (1.8)	-	1
	Unknown	2 (1.8)	-	1

Table 3. Results of SPTs according to the drugs involved and the drug tested.

		Drugs tested; positive cases/cases in which the test was performed (%)			
		Ciprofloxacin	Levofloxacin	Moxifloxacin	Total
Drugs involved; positive cases/cases in which the test was performed (%)	Ciprofloxacin	3/18 (16.7%)	2/9 (22.2%)	-	5/27 (18.5%)
	Levofloxacin	0/8	2/8 (25%)	4/5 (80%)	6/21 (28.6%)
	Moxifloxacin	1/16 (6.2%)	0/9	7/7 (100%)	8/34 (23.5%)
	Norfloxacin	0/1	0/1	-	0/2
	Pipemidic acid	2/2 (100%)	1/1 (100%)	1/1 (100%)	4/4 (100%)
	Unknown	1/1 (100%)	1/1 (100%)	1/1 (100%)	3/3 (100%)
	Total	7/46 (15.2%)	6/29 (20.7%)	13/14 (92.8%)	

Table 4. Results of BATs according to the drugs involved and the drug tested.

		Drugs tested; positive cases/cases in which the test was performed (%)						
		Ciprofloxacin	Levofloxacin	Moxifloxacin	Norfloxacin	Ofloxacin	Lomefloxacin	Total
Drugs involved; positive cases/cases in which the test was performed (%)	Ciprofloxacin	20/25 (80%)	9/19 (47.4%)	12/20 (60%)	5/6 (83.3%)	2/6 (33.3%)	4/6 (66.7%)	24/26 (92.3%)
	Levofloxacin	8/11 (72.7%)	5/11 (45.45%)	2/10 (20%)	1/1 (100%)	-	0/1	10/12 (83.3%)
	Moxifloxacin	26/34 (76.5%)	12/26 (46.2%)	20/32 (62.5%)	9/10 (90%)	8/10 (80%)	6/10 (60%)	32/35 (91.4%)
	Norfloxacin	1/1 (100%)	0/1	0/1	-	-	-	1/1 (100%)
	Ofloxacin	1/1 (100%)	0/1	1/1	-	-	-	1/1 (100%)
	Unknown	0/1	0/1	0/1	0/1	0/1	0/1	0/1
	Total	56/73 (76.7%)	26/59 (44.1%)	35/65 (53.8%)	15/18 (83.3%)	10/17 (58.8%)	10/18 (55.5%)	

Table 5. Results of DPTs performed according to the drugs involved and the drug tested.

		Drugs used in DPT; positive cases/cases in which the test was performed (%)		
		Ciprofloxacin	Levofloxacin	Moxifloxacin
Drugs involved in historical reaction; positive cases/cases in which the test was performed (%)	Ciprofloxacin	21/21	3/5 (60%)	-
	Levofloxacin	2/5 (40%)	5/5	0/1
	Moxifloxacin	5/8 (62.5%)	0/2	8/8
	Norfloxacin	1/1 (100%)	1/1 (100%)	-
	Unknown	-	1/1 (100%)	-

FIGURE LEGENDS

Figure 1. Algorithm proposed for the diagnosis of quinolone induced-HSRs. AGEP: Acute generalized exanthematous pustulosis. DPT: Drug provocation test. PT: Patch test. SJS: Stevens-Johnson syndrome. SPT: Skin prick test. TEN: Toxic epidermal necrolysis.

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TITLE: Clinical characterization and diagnostic approaches for patients reporting hypersensitivity reactions to quinolones

SHORT TITLE: Characterization and diagnosis of hypersensitivity reactions to quinolones.

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Total word count for the text: 3586 words.
Total word count for the Abstract: 250 words.

Conflict of interest: None of the authors has any conflict of interest or received any money for the present study. Research is part of their daily activities. All the authors had full access to all the data and can take responsibility for the integrity of the data and the accuracy of the data analysis.

Funding: The present study has been supported by the Institute of Health "Carlos III" of the Ministry of Economy and Competitiveness (grants cofounded by European Regional Development Fund (ERDF): RETIC ARADYALRD16/0006/0001, RD16/0006/0010, RD16/0006/0019 and RD16/0006/0030. I Doña is a Clinical Investigator (B-0001-2017) from Consejería de Salud of the Andalusian government, Junta de Andalucía. N Pérez-Sánchez holds a Rio Hortega research contract (CM17/0014), and E Barrionuevo a Juan Rodes research contract (JR18/00049), both from the Institute of Health "Carlos III", Spanish Ministry of Economy and Competitiveness (grants cofounded by European Social Fund, ESF).

ABSTRACT

Background: Quinolones are the second most frequent cause of hypersensitivity reactions (HSRs) to antibiotics. A marked increase in the number of patients with HSRs to quinolones has been detected.

Objective: To describe the clinical characteristics of patients with HSRs to quinolones and present methods for their diagnosis.

Methods: Patients attending the allergy unit due to reactions suggestive of HSRs to quinolones were prospectively evaluated between 2005-2018. Diagnosis was achieved using clinical history, skin tests (STs), basophil activation tests (BATs), and drug provocation tests (DPTs) if ST and BAT were negative.

Results: We included 128 subjects confirmed as having HSRs to quinolones and 42 found to be tolerant. Anaphylaxis was the most frequent entity in immediate HSRs and was most commonly induced by moxifloxacin. Patients were evaluated a median of 150 days (interquartile range: 60-365) after the reaction. Of patients who underwent ST and BAT, 40.7% and 70% respectively were positive. DPT with a quinolone was performed in 48 cases, giving results depending on the culprit drug: when moxifloxacin was involved, 62.5% of patients gave a positive DPT to ciprofloxacin, whilst none reacted to levofloxacin. The risk of HSR was 96 times higher in subjects who reported moxifloxacin-induced anaphylaxis and 18 times higher in those reporting immediate reactions compared to clinical entities induced by quinolones other than moxifloxacin and non-immediate reactions.

Conclusions: The diagnosis of HSR to quinolones is complex. The use of clinical history is essential as a first step. BAT shows higher sensitivity than STs. DPTs can be useful for finding safe alternative quinolones.

What is already known about this topic?

Quinolones can induce hypersensitivity through several mechanisms, being the third most common drug associated with hypersensitivity, and the second most frequent drug inducing both IgE-mediated hypersensitivity and severe anaphylaxis. The optimal diagnostic approach remains a controversial topic.

What does this article add to our knowledge?

The risk of having quinolone hypersensitivity is higher for immediate reactions, particularly for moxifloxacin-induced anaphylaxis. The basophil activation test has a higher sensitivity than skin test. Drug provocation testing can be useful to identify safe alternative quinolones.

How does this study impact current management guidelines

We propose an algorithm for diagnosing quinolone-induced reactions, which should be classified according to the interval between drug intake and reaction onset, using a 6 hour threshold. The algorithm includes skin, basophil activation, and drug provocation tests as necessary.

KEYWORDS: Adverse drug reaction; Anaphylaxis; Basophil activation test; Ciprofloxacin; Drug provocation test; Hypersensitivity; Levofloxacin; Moxifloxacin; Quinolones; Skin tests.

ABBREVIATIONS: Acute generalized exanthematous pustulosis (AGEP); Basophil activation test (BAT); Drug provocation test (DPT); Fixed drug eruption (FDE); Hypersensitivity reaction (HSR); Immediate reaction (IR); Intravenous (IV); Maculopapular exanthema (MPE); Non-immediate reaction (NIR); Skin prick test (SPT).

INTRODUCTION

Quinolones are antibiotics that are commonly prescribed for their effectiveness against Gram (+) and Gram (-) bacteria[1-3]. Adverse effects occur in 2-10% of people taking quinolones, however most of them are mild, mainly affecting the gastrointestinal or central nervous systems[3,4]. Quinolones can induce hypersensitivity reactions (HSRs)[5] through IgE-mediated reactions (immediate reactions, IRs) and T-cell dependent reactions (non-immediate reactions, NIRs)[6,7]. In addition, quinolones may also cause HSRs in drug-naïve patients[8,9]. A mechanism of mast cell activation via occupation of the human Mast-related G-protein receptor X2 (MRGPRX2) has been described for IRs[10,11] occurring in patients without previous exposure to quinolones[12].

Although the absolute risk of an HSR related to quinolones is low (44.0 (95% CI: 34.8–53.3) emergency department visits/100,000 prescriptions)[8], quinolones are the third most frequent drug associated with HSRs[13] in general, and the second most frequent in IgE-mediated HSRs. They are also the second most frequent cause of alert activation for antibiotic allergy in electronic hospital records[14] and severe drug-induced anaphylaxis[13,15]. In recent years, an increase in the percentage of patients with HSRs to these drugs has been detected, ranging from 0.54% in 2005 to 6.85% in 2010[13]. This is likely due to their increased prescription over the last decades[16]. The incidence of anaphylaxis induced by quinolones has been estimated to be 1.8–23 per 10 million days of treatment[17,18] and the prevalence of cutaneous adverse reaction to be 0.09%[19]. Quinolones are also one of the main triggers of acute generalized exanthematous pustulosis (AGEP), photosensitivity and vasculitis[20].

HSRs to quinolones appear in an important percentage of patients (23%) previously diagnosed as allergic to betalactams: in fact, betalactam allergic patients have a 17 times higher risk of reacting to quinolones than those non-allergic. This represents an important health problem as it greatly decreases therapeutic options availables[16]. In a large study of inpatients with common infections requiring antibiotic treatment, quinolone allergy occurred in 5.4% of patients who were already sensitive to betalactams[21], leading to important restrictions for antibiotic prescription and subsequently poor prognosis of their infections.

The optimal diagnosis of quinolone HSRs is still a matter of debate. The value of skin tests (STs) is uncertain, and they have shown false positive results when quinolones are tested at high concentration[22-25]. The presence of specific IgE to quinolones has been reported using the sepharose radioimmunoassay, with a sensitivity of 54.5%[26]. The

basophil activation test (BAT) has shown promising results for the diagnosis of patients with IRs to quinolones[27-29]. However, other studies have contradicted these findings[30,31]. The gold standard, therefore, is the drug provocation test (DPT). However, this is not free of risk and not advisable in cases where the reaction might be severe.

The aim of this study was to describe the clinical characteristics of a large group of patients with quinolone-induced HSRs and present methods for their diagnosis.

METHODS

Patients

We prospectively evaluated patients with symptoms suggestive of HSR to quinolones that had been referred to the Allergy unit of the University Regional Hospital of Málaga, University Hospital of Salamanca, and of the University Hospital La Fe of Valencia over a period of 13 years (2005-2018).

Inclusion criteria. Patients ≥14 years-old in whom the allergological study was completed were included and classified in two groups: A) Patients confirmed as having HSRs to quinolones (by positive STs, BATs or DPTs); and B) Patients confirmed as being non-allergic (tolerant) as they tolerated a DPT with the suspected culprit quinolone.

Exclusion criteria. Patients <14 years-old; patients in whom the allergological study was not completed so that the diagnosis could not be confirmed as being neither allergic nor tolerant to quinolones: pregnant or breastfeeding patients; patients taking beta-blockers or ACE inhibitors or with contraindications for epinephrine administration; patients who had acute infections and/or underlying cardiac, hepatic or renal diseases that contraindicated DPTs; and subjects with psychosomatic disorders.

Clinical history

Patients were asked about their reaction symptoms[32], the interval between drug intake and reaction onset, the number of episodes, the interval between their last reaction and the study, and the presence of other underlying diseases. If a reported reaction occurred within 6 hours after quinolone intake, the reaction was classified as IR; when this interval was longer, it was considered an NIR[5,32].

Skin testing

For reactions suggestive of an IR, skin prick tests (SPTs) were carried out as described[33] using ciprofloxacin (at 0.02 and 0.2 mg/ml), levofloxacin (at 0.05 and 0.5 mg/ml), and moxifloxacin (one tablet of 400 mg suspended in NaCI). Intradermal tests were not performed to avoid false positive results as non-specific histamine release by quinolones has been reported[34,35].

For reactions suggestive of a NIR, patch tests (PTs) were carried out and evaluated as described[33] by mixing powdered quinolone (ciprofloxacin, levofloxacin, and moxifloxacin) in petrolatum at 30% w/w.

Basophil activation test

In patients with a suspected IR, BATs were performed as described previously[27], using ciprofloxacin (2 and 0.2 mg/ml), levofloxacin (4 and 2 mg/ml), moxifloxacin (2, 0.2 and 0.1 mg/ml), norfloxacin (2, 0.2 and 0.1 mg/ml), ofloxacin (4, 2, 0.2 and 0.1 mg/ml), and lomefloxacin (4, 2, 0.2 and 0.1 mg/ml).

Drug provocation test

DPTs with ciprofloxacin, levofloxacin, and moxifloxacin were performed in a single blind manner if skin tests and BATs were negative[36]: placebo capsules were given at different times on the first day; increasing doses of quinolones were administered orally at intervals of 60 min (5, 20, 100 mg for ciprofloxacin and levofloxacin; 5, 30, 65 mg for moxifloxacin) on the second day. If these did not produce a reaction, three further doses of quinolones were given on the third day: 125, 125, 250 mg (accumulative dose 500 mg) for ciprofloxacin and levofloxacin; 100, 100, 200 mg (accumulative dose 400 mg) for moxifloxacin. The three test days were separated by 1 week. If cutaneous and/or respiratory symptoms or alterations in vital signs appeared, the procedure was stopped and the symptoms were evaluated and treated. If no symptoms appeared during graded challenge, the therapeutic dose was achieved. This was then followed by taking the full dose at home, starting 24 hours after the graded challenge. Before beginning the DPT procedure, patients were stable and their forced expiratory volume in 1s had to be at least 80% of the predicted value, with an absolute volume of at least 1.5 L. Medications were stopped before DPT according to international guidelines[36].

Statistical analysis

Data analysis was performed using Chi-square analysis to test differences in nominal variables between groups, the Fisher test was used when there were no criteria for using the Chi-square test and the Mann–Whitney test was used for quantitative variables. All reported p values represented two-tailed tests, with values <0.05 considered statistically significant. A logistic regression analysis was performed to establish the characteristics associated with the diagnosis of HSR or tolerance to quinolones and with the diagnosis of immediate anaphylaxis. The following variables were analysed: gender, age, time interval between drug intake and the onset of the reaction, symptoms experienced, drugs involved, time interval between drug reaction and study, and number of episodes.

The study was conducted according to the principles of the Declaration of Helsinki. All the participants were informed orally about the study and signed the corresponding informed consent.

RESULTS

A total of 612 patients with a clinical history suggestive of an HSR to quinolones were evaluated. Of these, full diagnosis could not be achieved for 442 patients: 361 patients that gave a negative ST and negative BAT could not undergo DPT to quinolones due to age, comorbidities or because it was contraindicated due to the potential severity of the reaction; 78 did not give consent for the allergological tests (STs, BAT and/or DPTs); and 3 were excluded due to pregnancy. For the remaining 170 patients a full diagnosis could be achieved: 128 were confirmed as having HSRs to quinolones and 42 as non-allergic (tolerant) to quinolones.

Clinical data of the subjects included in the study

The 170 included subjects with confirmed diagnosis had a median age of 53 [interquartile range: 40–63.25] years, and 126 (74.1%) were female. The majority of cases reported only one previous episode induced by quinolone intake, except for 2 cases who reported 2 previous IRs. As such, the patients included in the study reported a total of 172 previous reactions: 120 IRs and 52 NIRs, with the percentage of IRs higher in those confirmed as having HSRs compared to the tolerant group (73.8% vs 19%; $p<0.0001$) (Table 1). Most reported reactions were induced by oral quinolones (142; 82.5%), the rest by intravenous route (30;17.4%). In terms of the symptoms of reported reactions, the percentage of anaphylaxis reactions was higher in subjects confirmed as having HSR ($p<0.0001$); whereas urticaria ($p=0.0004$), local reaction at the site of IV administration ($p=0.0001$) and MPE ($p=0.03$) were more frequently report by subjects that were found to be as tolerant (Table 1). Moxifloxacin was the most frequent culprit quinolone in patients with confirmed HSRs; ciprofloxacin was more frequent in subjects confirmed as tolerant ($p<0.0001$ and $p=0.001$, respectively) (Table 1). In subjects confirmed as having HSRs, most cases of anaphylaxis were induced by moxifloxacin (52.9%; $p=0.002$); urticaria and angioedema were mostly induced by ciprofloxacin (48.8% and 66.7%, respectively), although these differences was not found to be statistically significant (Table E1). For those found to be tolerant, ciprofloxacin was the most frequent cause of both urticaria and angioedema (69.2% and 66.7%, respectively), as well as of local reactions at the site of IV administration (66.7%) ($p>0.05$) (Table E1).

The logistic regression analysis showed that the risk of being confirmed as HSR was higher for cases who reported moxifloxacin-induced anaphylaxis (OR: 96.16; CI: 6.172-Inf; $p=0.009$) and for those reporting IRs (OR: 18.856; CI: 5.196-271.449; $p<0.0001$)

compared to cases who reported other symptoms induced by other quinolones and NIRs. Moreover, the risk for being confirmed as HSR decreased when ciprofloxacin was the culprit (OR: 0.107; CI: 0.002-0.741; p=0.04) and the symptoms reported were MPE, FDE, urticaria, angioedema (OR: 0.053; CI: 0-0.452; p=0.03), or a local reaction at the site of IV administration (OR: 0.001; CI: 0-0.016; p=0.0006). No significant associations were found for the other variables, and there were no interactions between variables.

Analysis of the patients confirmed as suffering HSRs to quinolones

In patients confirmed as having HSRs, a total of 112 reported reactions (73.8%) were IRs and 18 (26.2%) NIRs. No differences were found when comparing age, sex, atopy, allergen sensitization and underlying diseases between IR and NIR groups (data not shown).

Anaphylaxis was the most frequent reported symptom among IRs ($p<0.0001$) and urticaria among NIRs ($p>0.05$) (Table 2). It is of note that the 7 (25%) of patients reporting reactions within the interval of 1-6 hours showed symptoms compatible with anaphylaxis. Moxifloxacin was the most frequent quinolone involved in IRs and ciprofloxacin in NIRs (41.1% and 38.9%, respectively) (Table 2). Anaphylaxis was induced primarily by moxifloxacin in IRs (52.9%; OR=2.935 (IC:1.418-6.075), p=0.003) whereas most urticaria and angioedema was induced by ciprofloxacin (52.9% and 75%, respectively). Considering NIRs, moxifloxacin was the culprit in most cases reporting urticaria (42.8%) and ciprofloxacino in angioedema reporting-cases (50%) (Table E2). The time interval between intake and onset of the reaction was shorter when the drug was administered by an IV route compared to the oral route (5 [IR: 5-10] minutes vs 30 [IR: 15-60] minutes, p=0.005). This comparison was also statistically significant when ciprofloxacin was the culprit (IV route: 5 [5-10] minutes; oral route: 30 [18.7-165] minutes; p=0.01) (Table E3).

Methods used for diagnosis

The median time interval between the reaction and the study was 150 days [interquartile range: 60-365] (mean: 560.3 days, SD: 1028.4 days). No differences were found between IRs and NIRs. STs were performed on 54 subjects, BATs on 76, and DPTs on 48. No differences were found when comparing the clinical characteristics of patients undergoing the different tests (data not shown).

Skin tests

SPTs were performed on 48 patients and PTs on 6, of which 22 were positive: 20 SPTs and 2 PTs. Of the positive SPTs, 13 (43.33%) were positive to moxifloxacin, 7 (8.53%) to ciprofloxacin, and 6 (9.83%) to levofloxacin (Table 3). When ciprofloxacin was the suspected culprit drug, SPTs to ciprofloxacin were positive in 16.7% of the tests and levofloxacin in 22.2%; when levofloxacin was the suspected culprit, 25% of SPTs were positive to levofloxacin and 80% to moxifloxacin. Finally, when the suspected culprit was moxifloxacin, 100% of SPTs were positive to moxifloxacin and 6.2% to ciprofloxacin (Table 3). Regarding the symptomatology of the reported reactions, the highest percentage of positive ST results was found for anaphylaxis (53.8%), followed by urticaria (33.3%). Although the interval between the last quinolone-induced reaction and the study was shorter in patients with positive STs compared to negative, no statistical difference was found (90 [interquartile range: 60-240] vs 120 [interquartile range: 60-172.5] days; p=1).

Basophil activation test

The BAT was positive in 68 (89.5%) of cases. A total of 56 (76.7%) cases were positive to ciprofloxacin, 35 (53.8%) to moxifloxacin, 26 (44.1%) to levofloxacin, 15 (83.3%) to norfloxacin, 10 (58.8%) to ofloxacin and 10 (55.5%) to lomefloxacin (Table 4). When ciprofloxacin was the culprit, BAT to ciprofloxacin was positive in 80%, to moxifloxacin in 60% and to levofloxacin in 47.4%; when levofloxacin was the culprit, BAT was positive to ciprofloxacin in 72.7%, to levofloxacin in 45.4% and to moxifloxacin in 20%; finally, when the culprit drug was moxifloxacin, BAT was positive to ciprofloxacin in 76.5%, to moxifloxacin in 62.5% and to levofloxacin in 46.2% (Table 4). BAT was positive in 48 out of 49 (97.9%) cases reporting anaphylaxis, in 13 out of 18 (72.2%) cases of urticaria and in 5 (100%) cases of angioedema. Although the interval between the historical quinolone reaction and whether the patients were found to be allergic or not in the study was shorter in patients who gave a positive BAT compared to negative, no statistical differences were found (150 [interquartile range: 60-365] vs 395 [interquartile range: 60-1003.7] days; p=0.9909).

Drug provocation test

We performed 58 DPTs in 48 patients. A total of 34 DPTs with the culprit quinolone were carried out in cases with negative ST and BAT, all of which were positive: 23 in IR (16 with ciprofloxacin, 3 with levofloxacin, and 4 with moxifloxacin) and 11 in NIR (5 with ciprofloxacin, 2 with levofloxacin, and 4 with moxifloxacin). When the benefit

was considered to outweigh the risk, we carried out DPTs with an alternative quinolone in 24 cases, this was positive for 13 of these (11 IR and 2 NIR) (Table 5). When ciprofloxacin was the culprit, DPT to levofloxacin was positive in 60%; when levofloxacin was the culprit, DPT was positive to ciprofloxacin in 40%; when the culprit drug was moxifloxacin, DPT was positive to ciprofloxacin in 62.5%, and no case reacted to levofloxacin (Table 5). In all cases DPTs with quinolones induced mild symptoms (pruritus and wheals localized on different parts of the body) that disappeared 1-48 hours after administering antihistamine and corticosteroid treatment.

DISCUSSION

Although hypersensitivity reactions (HSRs) to quinolones represent an important health problem[21], no large-scale study of patients suffering from them exists. To our knowledge, this is the largest published series of HSRs to quinolones to date. In our study, more than the 50% of patients reported anaphylaxis, most of whom suffered from immediate reactions (IRs), in agreement with previously published short series[24,26,27,30,34]. Moreover, data suggests that the risk of an HSR is different depending on the quinolone. Analyses of spontaneous reports implicate moxifloxacin triggers anaphylaxis in a higher proportion of cases than levofloxacin or ciprofloxacin[8,9,37], which is in line with our results. Indeed, the risk of experiencing anaphylaxis was 2.2-fold higher when moxifloxacin was the culprit, which agrees with previously published data[8,9,37]. This may be due to the expanded use of quinolones or increased immunogenicity to newer quinolones.

The interval between drug intake and the appearance of symptoms is crucial for evaluating allergic reactions to drugs. Historically, reactions occurring less than one hour after drug intake are considered IRs, and those occurring after an hour are considered NIRs[5,38]. The former are thought to be induced by an IgE-mediated response, although an alternative non-IgE dependent mechanism may also be involved[10,11]. For the latter, the underlying mechanism remains a matter of debate, especially for those with a time interval of 1 to 6 hours after drug intake[39-41]. For betalactam antibiotics, the mechanism has been proposed to be non-IgE dependent, as some evidence suggests that these reactions are T-cell mediated[40-41]. On the other hand, for metamizole, a study of reactions occurring 1–8 hours after intake using basophil activation testing support an IgE-mediated[42]. However, to our knowledge, this mechanism has not yet been studied for quinolones. In this study, we have observed that around 25% of patients reported anaphylaxis 1 hour after quinolone intake, and more than 40% of them showed positive

results via BAT or SPT, suggesting that an IgE mechanism is likely. The interval between drug administration and reaction onset may be related to the production of as-yet unidentified metabolites and the route of administration. However, most patients in our study took the quinolone orally and no differences could be found in terms of administration route when considering drugs involved and symptoms reported. As such, we would suggest that the classification of reactions as IR or NIR based solely on a 1 hour cut-off does not sufficiently reflect the extension of the pathophysiology of the reactions.

The diagnosis of HSRs to quinolones is still a matter of debate. A detailed clinical history is crucial as a first approach. We found that the chance of being confirmed as having an HSR to quinolones was 96 times higher in patients who reported moxifloxacin-induced anaphylaxis and 18 times higher in those reporting IRs. This risk decreased when ciprofloxacin was the culprit and the symptoms experienced in the reported reaction were MPE, FDE, urticaria, angioedema or a local reaction at the site of the administration of the drug. Concerning STs, there is controversy regarding their utility. Some authors consider they are useful, with a sensitivity of 71%, specificity of 86%, and positive and negative predictive values of 50% and 94%, respectively having been reported previously[24]. However, other studies suggest that STs are not valid because they can produce false-negatives[34,35], potentially missing important reactions and putting patients at risk, moreover they can also produce a large number of false-positive results when tested at high concentrations, which is attributed to non-specific histamine release by quinolones due to mast cell activation[35,43-48]. We decided not to perform intradermal tests in our patients based on this consideration. In our study, we found a low sensitivity for STs in general, although it was higher for severe reactions (anaphylaxis) and when levofloxacin and ciprofloxacin were the culprits.

We found the BAT to be useful for the diagnosis of patients with IRs to quinolones[27-29]. However, other studies have shown contradictory results[30,31]. Here, BAT gave a higher percentage of positive results than STs, agreeing with previous studies[27,28]. This is important, because if BATs can be used to confirm diagnosis instead of DPTs in some cases, this will reduce patient risk. This is particularly useful here, given that the most common clinical entity induced by quinolones is anaphylaxis.

Although cross-reactivity among quinolones remains a controversial issue, DPTs could be useful to find safe alternative quinolones. A high degree of cross-reactivity has been reported between the first- and second- generation quinolones[22]. Regarding the second

generation, cross-reactivity does not always occur within this group[43,45,49], which may be due to the production of different metabolites. The same phenomenon can occur for the newer (moxifloxacin) and the second- (ciprofloxacin) and third-generation (levofloxacin) quinolones[50-52]. A low degree of cross-reactivity has been found between levofloxacin and ciprofloxacin[34]. In our study, 60% of the patients who reported reactions induced by levofloxacin tolerated ciprofloxacin in DPT and 40% of cases tolerated levofloxacin when the reactions were induced by ciprofloxacin. DPT with levofloxacin was carried out for 2 cases who reported moxifloxacin-induced reactions, with neither patient experiencing an adverse reaction. Based on the data obtained from our large series of cases, we propose an algorithm for the diagnosis of quinolone-induced HSRs, as described in Figure 1.

A limitation of this study is the high percentage of patients for whom we were not able to confirm the diagnosis due contraindication or patient refusal. This could be the reason why the number of cases confirmed as tolerant in our series is low. However, despite this, our results show relevant differences in clinical characteristics comparing tolerant and cases confirmed as HSRs, highlighting the importance of a detailed clinical history as an initial approach for diagnosis. Another limitation of our study is that ST, BAT, and DPT could not be performed for all patients and with all quinolones, that PT was carried out at a 30% dilution in petrolatum which could increase the rate of false negative results, and that the time interval between the reaction and the allergy evaluation was not uniform in all patients. However, our aim was to describe the role and utility of the different diagnostic methods performed in a large group of patients in real allergological practice, not finding differences in the clinical characteristics when comparing groups of patients based on results for ST, BAT, and DPT.

The accurate diagnosis of quinolone-induced HSRs is an important issue not only due to their frequency, as described above, but also due to the fact that an important percentage of patients that report quinolone-induced HSRs report previous reactions to betalactams, drastically reducing their therapeutic options[16]. Referring patients with quinolone-induced HSRs for a full allergological evaluation is crucial to confirm or dismiss their reported allergy.

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Table 1. Clinical data for the reactions reported by the subjects included in the study, comparing cases confirmed as having HSRs to quinolones and those confirmed as tolerant to these drugs. AE: Angioedema. FDE: Fixed drug eruption. HSR: Hypersensitivity reaction. MPE: Maculopapular exanthema.

		HSR n=130	Tolerant n=42	p
Historical reaction symptoms; n (%)	Anaphylaxis	70 (53.8)	-	<0.0001
	Urticaria	41 (31.5)	26 (61.9)	0.0004
	AE	12 (9.2)	6 (14.3)	0.3522
	FDE	4 (3.1)	-	0.5732
	MPE	3 (2.3)	4 (9.5)	0.03
	Local reaction at the site of intravenous administration	-	6 (14.3)	0.0001
Drugs involved in historical reactions; n (%)	Ciprofloxacin	49 (37.7)	28 (66.7)	0.001
	Levofloxacin	21 (16.2)	10 (23.8)	0.2618
	Moxifloxacin	52 (40)	2 (4.8)	<0.0001
	Norfloxacin	3 (2.3)	2 (4.8)	0.5967
	Ofloxacin	1 (0.8)	-	1
	Pipemidic acid	2 (1.5)	-	1
	Unknown	2 (1.5)	-	1

Time interval intake-reaction, median (IR) (min)		30 (11.25-60)	7200 (2880-8640)	<0.0001
Time interval intake-reaction ≤1h; n (%) Time interval intake-reaction >1h; n (%)		112 (73.8) 18 (26.2)	8 (19) 34 (81)	<0.0001
Administration route; n (%)	Oral Intravenous	109 (83.8) 21 (16.1)	33 (78.6) 9 (21.4)	0.4335
Number of episodes, median (IR)		1 (1-1)	1 (1-1)	0.08

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Table 2. Clinical data for the reported reactions in cases confirmed as HSRs to quinolones. AE: Angioedema. FDE: Fixed drug eruption. MPE: Maculopapular exanthema.

		Immediate n (%)	Non-immediate n (%)	p
Historical reaction symptoms; n (%)	Anaphylaxis	70 (62.5)	-	<0.0001
	Urticaria	34 (30.4)	7 (38.9)	0.4696
	AE	8 (7.1)	4 (22.2)	0.04
	FDE	-	4 (22.2)	0.0002
	MPE	-	3 (16.7)	0.002
Drugs involved in historical reaction; n (%)	Ciprofloxacin	42 (37.5)	7 (38.9)	0.9101
	Levofloxacin	17 (15.2)	4 (22.2)	0.451
	Moxifloxacin	46 (41.1)	6 (33.3)	0.5339
	Norfloxacin	2 (1.8)	1 (5.6)	0.3629
	Ofloxacin	1 (0.9)	-	1
	Pipemidic acid	2 (1.8)	-	1
	Unknown	2 (1.8)	-	1

Table 3. Results of SPTs according to the drugs involved and the drug tested.

		Drugs tested; positive cases/cases in which the test was performed (%)			
		Ciprofloxacin	Levofloxacin	Moxifloxacin	Total
Drugs involved; positive cases/cases in which the test was performed (%)	Ciprofloxacin	3/18 (16.7%)	2/9 (22.2%)	-	5/27 (18.5%)
	Levofloxacin	0/8	2/8 (25%)	4/5 (80%)	6/21 (28.6%)
	Moxifloxacin	1/16 (6.2%)	0/9	7/7 (100%)	8/34 (23.5%)
	Norfloxacin	0/1	0/1	-	0/2
	Pipemidic acid	2/2 (100%)	1/1 (100%)	1/1 (100%)	4/4 (100%)
	Unknown	1/1 (100%)	1/1 (100%)	1/1 (100%)	3/3 (100%)
	Total	7/46 (15.2%)	6/29 (20.7%)	13/14 (92.8%)	

Table 4. Results of BATs according to the drugs involved and the drug tested.

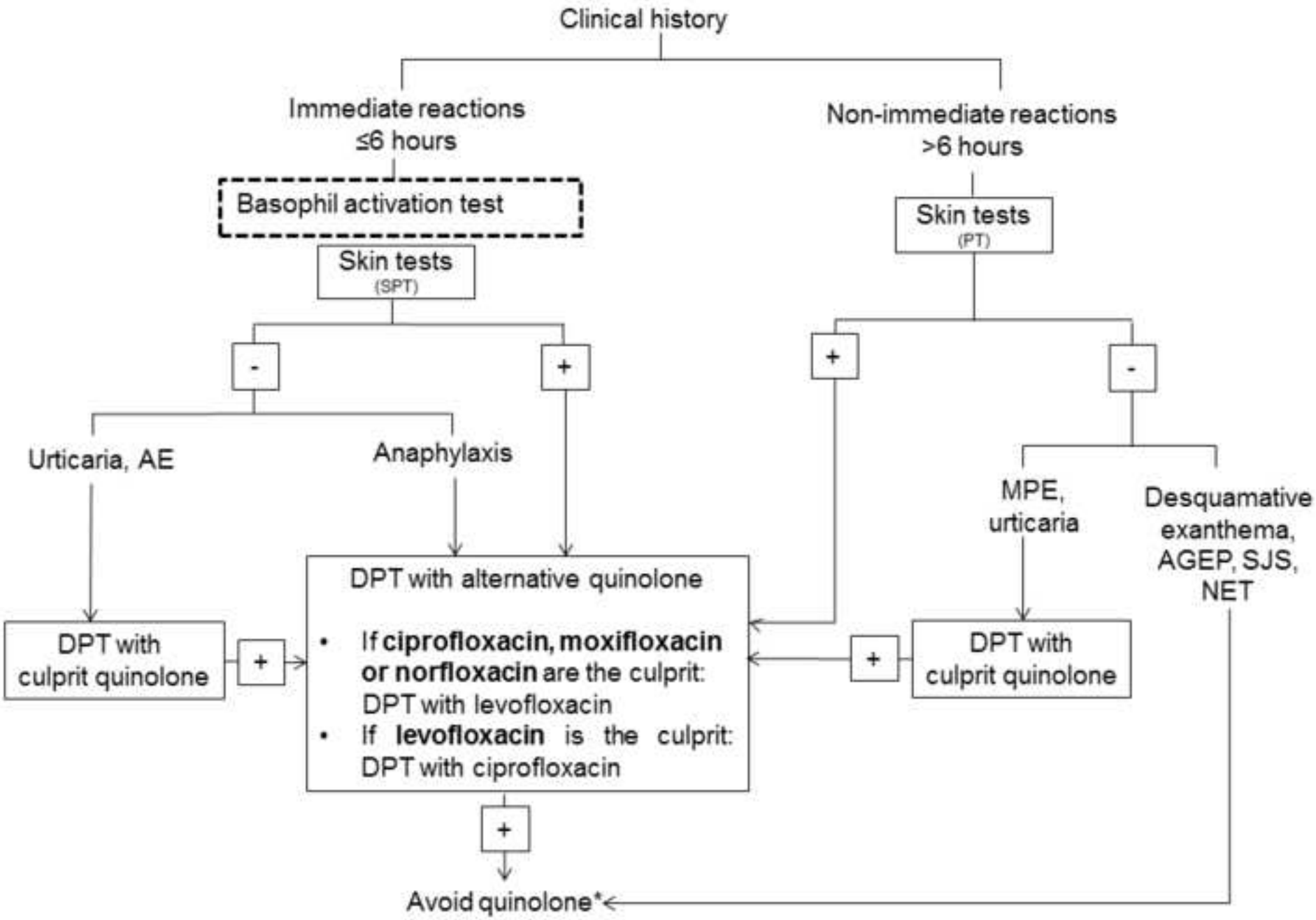
		Drugs tested; positive cases/cases in which the test was performed (%)						
		Ciprofloxacin	Levofloxacin	Moxifloxacin	Norfloxacin	Ofloxacin	Lomefloxacin	Total
Drugs involved; positive cases/cases in which the test was performed (%)	Ciprofloxacin	20/25 (80%)	9/19 (47.4%)	12/20 (60%)	5/6 (83.3%)	2/6 (33.3%)	4/6 (66.7%)	24/26 (92.3%)
	Levofloxacin	8/11 (72.7%)	5/11 (45.45%)	2/10 (20%)	1/1 (100%)	-	0/1	10/12 (83.3%)
	Moxifloxacin	26/34 (76.5%)	12/26 (46.2%)	20/32 (62.5%)	9/10 (90%)	8/10 (80%)	6/10 (60%)	32/35 (91.4%)
	Norfloxacin	1/1 (100%)	0/1	0/1	-	-	-	1/1 (100%)
	Ofloxacin	1/1 (100%)	0/1	1/1	-	-	-	1/1 (100%)
	Unknown	0/1	0/1	0/1	0/1	0/1	0/1	0/1
	Total	56/73 (76.7%)	26/59 (44.1%)	35/65 (53.8%)	15/18 (83.3%)	10/17 (58.8%)	10/18 (55.5%)	

Table 5. Results of DPTs performed according to the drugs involved and the drug tested.

		Drugs used in DPT; positive cases/cases in which the test was performed (%)		
		Ciprofloxacin	Levofloxacin	Moxifloxacin
Drugs involved in historical reaction; positive cases/cases in which the test was performed (%)	Ciprofloxacin	21/21	3/5 (60%)	-
	Levofloxacin	2/5 (40%)	5/5	0/1
	Moxifloxacin	5/8 (62.5%)	0/2	8/8
	Norfloxacin	1/1 (100%)	1/1 (100%)	-
	Unknown	-	1/1 (100%)	-

FIGURE LEGENDS

Figure 1. Algorithm proposed for the diagnosis of quinolone induced-HSRs. AGEP: Acute generalized exanthematous pustulosis. DPT: Drug provocation test. PT: Patch test. SJS: Stevens-Johnson syndrome. SPT: Skin prick test. TEN: Toxic epidermal necrolysis.



*When a given quinolone is the only therapeutic option available, desensitization could be indicated

Table E1. Analysis of the drug involved in each reaction according to the symptoms reported for cases confirmed as having HSR to quinolones and those confirmed as tolerant. The percentages are given for the symptoms reported (columns). AE: Angioedema. FDE: Fixed drug eruption. HSR: Hypersensitivity reaction. IV: Intravenous. MPE: Maculopapular exanthema.

	HSR					Tolerant			
	Anaphylaxis	Urticaria	AE	FDE	MPE	Urticaria	AE	MPE	Local reaction at IV site administration
Ciprofloxacin	18 (25.7)	20 (48.8)	8 (66.7)	1 (25)	2 (66.7)	18 (69.2)	4 (66.7)	2 (50)	4 (66.7)
Levofloxacin	12 (17.1)	6 (14.6)	2 (16.7)	-	1 (33.3)	4 (15.4)	2 (33.3)	2 (50)	2 (33.3)
Moxifloxacin	37 (52.9)	11 (26.8)	2 (16.7)	2 (50)	-	2 (7.7)	-	-	-
Norfloxacin	-	2 (5.8)	-	1 (25)	-	2 (7.7)	-	-	-
Ofloxacin	1 (1.4)	-	-	-	-	-	-	-	-
Pipemidic acid	1 (1.4)	1 (2.4)	-	-	-	-	-	-	-
Unknown	1 (1.4)	1 (2.4)	-	-	-	-	-	-	-
p	0.002	0.2366	0.3949	0.2391	0.4201	0.2759	0.8126	0.5244	0.8126

Table E2. Quinolones involved in the reported reactions according to the symptoms experienced in cases confirmed as HSR to quinolones. . AE: Angioedema. FDE: Fixed drug eruption. MPE: Maculopapular exanthema.

	Immediate; n (%)			Non-immediate; n (%)			
	Anaphylaxis	Urticaria	AE	Urticaria	AE	FDE	MPE
Ciprofloxacin	18 (25.7)	18 (52.9)	6 (75)	2 (28.6)	2 (50)	1 (25)	2 (66.7)
Levofloxacin	12 (17.1)	4 (11.8)	1 (25)	2 (28.6)	1 (25)	-	1 (33.3)
Moxifloxacin	37 (52.8)	8 (23.5)	1 (25)	3 (42.8)	1 (25)	2 (50)	-
Norfloxacin	-	2 (5.9)	-	-	-	1 (25)	-
Ofloxacin	1 (1.4)	-	-	-	-	-	-
Pipemidic acid	1 (1.4)	1 (2.9)	-	-	-	-	-
Unknown	1 (1.4)	1 (2.9)	-	-	-	-	-
p	0.001	0.01	0.3937	0.6894	1	0.2154	0.511

Table E3. Comparison of time interval between drug intake and the onset of the reaction for oral and intravenous routes, according to the quinolone involved and the symptoms reported in the reaction. AE: Angioedema. FDE: Fixed drug eruption. MPE: Maculopapular exanthema.

		Time interval between drug intake and reaction onset mediane (IR) minutes		
		Oral	Intravenous	p
Drugs involved	Ciprofloxacin	30 (18.7-165)	5 (5-10)	0.01
	Levofloxacin	30 (17.5-240)	-	-
	Moxifloxacin	30 (10-30)	-	-
	Norfloxacin	27.50 (26.2-28.75)	-	-
	Ofloxacin	17.5 (11.2-23.75)	-	-
	Pipemidic acid	70 (45-95)	-	-
	Unknown	20 (15-25)	-	-
Symptoms experiencedin reported reactions	Anaphylaxis	30 (10-30)	7.5 (5-10)	0.04
	Urticaria	30 (30-120)	5 (5-5)	0.1479
	AE	600 (240-2880)	17.5 (11.2-23.7)	0.118
	FDE	1740 (1170-2310)	-	-
	MPE	720 (660-1800)	-	-